University of Alberta

The Effect of Inflammation on Propranolol Pharmacokinetics and Pharmacodynamics

by

Micheal Said Guirguis

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in

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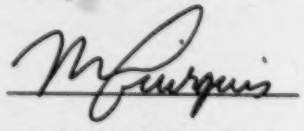

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Date: Feb 19, 2002

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Effect of Inflammation on Propranolol Pharmacokinetics and Pharmacodynamics submitted by Micheal Said Guirguis in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

in Pharmaceutical Sciences

Dr. F. Jamali

Dr. Y.K. Tam

Dr. R. Lewanczuk

Dr. Ayman El-Kadi

Dr. A. S. Russell

Dr. P. K. F. Yeung

Date: Feb 13, 2002

Abstract

The objectives of this study were to primarily confirm the effects of inflammation on propranolol pharmacokinetics and determine the effects on propranolol pharmacodynamics. Secondly, to examined the effect of anti-cytokine therapy (i.e., infliximab, an anti-TNF agent) on propranolol pharmacokinetics and pharmacodynamics in adjuvant arthritis model. Finally, we endeavored to determine if reduced blood pressure control associated with nonsteroidal anti-inflammatory therapy is linked to the rate of gastrointestinal toxicity.

Using the adjuvant arthritic rat model, Sprague Dawley rats were used to determine the effects inflammation on propranolol pharmacokinetics and pharmacodynamics. Reduction in heart rate and PR interval and were used for measuring pharmacodynamics. Dose effect curves for adjuvant arthritic and control rats, revealed that inflammation brings about substantial reduction in propranolol potency. A simultaneous pharmacokinetic and pharmacodynamic study revealed surprisingly that this reduced response was accompanied by a several fold increase in propranolol plasma concentrations.

The effect of anti-TNF therapy was examined in a simultaneous pharmacokinetic and pharmacodynamic pilot study. Adjuvant arthritic rats again showed significant reduction in propranolol potency in the presence of highly elevated plasma concentrations. Five days after anti-TNF therapy reassessment of propranolol pharmacokinetics and pharmacodynamics revealed a complete reversal of the effects on propranolol potency.

The effect of NSAID therapy on cardiac indices and propranolol potency was determined by examining a variety of NSAIDs with differing degrees of GI toxicity. Baseline cardiac indices were monitored which included heart rate, PR interval and blood pressure. Propranolol potency was monitored, with respect to heart rate, PR interval and blood pressure. Treatment with flurbiprofen and indomethacin resulted in a significant change in baseline PR interval. This was not observed following treatment with celecoxib, a selective cyclooxygenase-2 inhibitor. As well, if gastrointestinal toxicity of flurbiprofen was prevented with metronidazole co-treatment, no change in baseline PR interval was observed. An examination of propranolol potency showed that both flurbiprofen and indomethacin therapy caused significant reduction in propranolol potency. Celecoxib and co-treatment with metronidazole resulted in no change in propranolol potency. These results demonstrate a possible link between the incidence of gastrointestinal toxicity of NSAIDs and reduction in antihypertensive effectiveness.

Hypothesis

Knowledge
Science, source of knowledge?

Life
Science, to understand life.

Humanity
Science, brought to humility.

Micheal Said

Dedication

To my parents Said and Aspasia and my brother Mark through their support I was able to fulfill my dreams, and to my lovely wife Lisa for being at my side at the darkest and brightest times

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List of Abbreviations

AA	adjuvant Arthritis
AUC	area under the concentration curve
AUEC	area under the effect curve
Bid	twice daily dosing
β T ½	terminal half life
bpm	beats per minute
CYP 450	cytochrome P 450
EC_{50}	concentration required to achieve 50 % of maximum effect
E_{max}	the maximum achievable effect
GI	gastrointestinal
i.p.	intraperitoinal
IL	interleukin
NO	nitric oxide
NSAID	non-steroidal anti-inflammatory drug
PO	Oral dosing
PK/PD	linked pharmacokinetic and pharmacodynamic
TNF	tumor necrosis factor

Chapter I: Introduction

An estimated 4 million Canadians suffer from arthritis. The majority of them are over 45 years of age. Due to the nature of the disease, arthritic patients are on a variety of long-term anti-inflammatory medications and are thus likely to suffer side effects, toxicities and drug interactions.[1] Studies have shown that patients suffering from arthritis, in all its forms, are more likely to suffer from cardiac disorders, such as myocardial infarction [2] and angina. [3] As well, clinicians have observed a reduced ability to control blood pressure in arthritis patients.[4] As of yet, no researcher has examined the reasons for these observations.

Since the time of the pharaohs, scientists have been aware of the unique pathophysiological status of patients suffering from arthritis. Thus it came as no surprise to some that this included modifications in xenophobic metabolism. In a serendipitous discovery, Babb *et al.* found that patients with elevated erythrocyte sedimentation rate (a general indicator of inflammation) had reduced propranolol clearance.[5-8] Further studies by Belpaire and colleagues, using isolated hepatocytes from rats exposed to turpentine (an inducer of mild inflammation), found that in the presence of inflammation propranolol metabolism is significantly reduced. They concluded that inflammation triggers a process that significantly alters propranolol metabolism.[9] [10-12]The mechanism by which this process occurs continues to be elucidated by many researchers.

As stated earlier rheumatic patients have been found to have a significantly increased incidence of cardiovascular disease. The potential effects of chronic

systemic inflammation on the function of the cardiovascular system, cannot be understated. In an early study looking at the effect of inflammatory mediators (i.e., cytokines) on β-adrenergic receptors, researchers observed substantial changes in receptor function.[13] Using T-lymphocytes the researchers looked at the effect of 18 h incubation with interleukin-2 (IL-2, an inflammatory cytokine) on β_2-adrenergic receptor density and activity. Researchers determined that although there was an initial increase in receptor density this was followed by a 50% reduction. Surprisingly though, they found that the increase in receptor number coincided with reduced receptor response to agonists, demonstrating modification in receptor function.[13] Thus, even short-term exposure to inflammatory mediators brings, about substantial changes to β-adrenergic receptor function.

Further complicating the understanding of physiological changes occurring in rheumatoid arthritis patients is their long-term use of anti-inflammatory agents. Acting on a variety of physiological process and causing a variety of side effects, these agents can include a wide variety of agents from methotrexate to nonsteroidal anti-inflammatory drugs (NSAIDs). With respect to blood pressure control though, NSAIDs use has long been contraindicated. In a meta-analysis of studies examining the interaction of NSAIDs and antihypertensives it was observed that not only do NSAIDs antagonize the activity of a variety of antihypertensives, they increased baseline blood pressure.[14]

Thus a variety of physiological processes, are affected in patients with inflammatory conditions. Inflammation brings about a significant increase in circulating inflammatory mediators (i.e., cytokines, nitric oxide), which are thought

to trigger significant changes in body function.[15] As of yet no one has tried to link the observations of clinicians with the discoveries of scientists to explain the unique pharmacokinetic and pharmacodynamic status of patients suffering from rheumatoid arthritis. The aim of this thesis was to investigate the effect of inflammation on the pharmacokinetics and pharmacodynamics of cardiovascular agents in the presence and absence of anti-inflammatory agents; using an arthritis rat model and propranolol, a stereotypical β-adrenergic receptor antagonist.

Propranolol Pharmacokinetics

Propranolol, a chiral molecule, is a nonspecific β-adrenergic receptor antagonist used initially for the treatment of hypertension and more recently for migraine prophylaxis. Administered as a racemate, propranolol is highly metabolized through the cytochrome P450 system (predominantly through CYP 2D6, CYP 2C9, and CYP 1A2) and is classified as a highly cleared drug; thus undergoing extensive liver first pass metabolism, which is highly dependent on cytochrome P450 function. These characteristics make it an ideal drug for assessing changes in first pass metabolism.[16]

Propranolol has a relatively short half-life ranging from 0.5 – 2 h, despite a large volume of distribution (3.3-5.5 L/kg).[16] Propranolol is highly bound to plasma α_1-acid glycoprotein and to a much lesser extent albumin. Total body clearance of the drug has been found to approach 1L/min after intravenous administration, approaching hepatic blood flow. Thus, propranolol hepatic extraction is highly dependent on blood flow. After oral administration its bioavailability has been

determined to range from 5-15 % and is highly variable. This variability is thought to occur due to inter-subject variation in cytochrome activity, as absorption is almost complete. Variability is also seen between different species, with rats having higher rates of clearance as compared to humans.[16] These interspecies differences are thought to be due to changes in cytochrome P450 content and hepatic blood flow.[17]

Liver metabolism

At low oral propranolol doses, metabolism is almost complete, with almost complete hepatic extraction. With increasing oral doses however, there is a non-linear increase in AUC, attributed to saturation of the primary metabolic pathways. This has also been observed, with multiple propranolol dosing where there is an unpredicted level of accumulation. Thus, propranolol metabolism can be characterized by low dose linear pharmacokinetics followed by saturated kinetics at higher oral doses.[16-20]

Major products of propranolol oxidative metabolism in mammals consist of 4-, 5-, and 7 hydroxy-proranolol and N-desisoproranolol. Major cytochrome P450 isozymes responsible for propranolol metabolism include CYP 2D6, CYP 2C9 and potentially CYP 1A2. In humans, propranolol 4-, and 5-hydroxylation are the major metabolites, with only trace levels of the 7-OH metabolite. This is in contrast to propranolol metabolism in the rat, where the 7-OH metabolite is the major metabolite. [21; 22]

Propranolol metabolism is stereoselective. This is clinically important because the S enantiomer is 60-100 times more potent then the R enantiomer. In humans, it has been found that the R enantiomer is more readily eliminated, while in rats the opposite holds true.[23] This is believed to be due to the species dependent stereoselective nature of the Phase I and Phase II metabolism.[23, 24]

Propranolol Pharmacodynamics

Propranolol is a β-adrenergic receptor antagonist, which is used for a variety of therapeutic purposes. The β-adrenergic receptor is part of the sympathetic system. In the presence of agonists such as epinephrine and norepinephrine, an in increase heart rate, blood pressure and shortening of the PR interval is triggered. Therefore, the blockade of the β-adrenergic receptor by propranolol results in a reduction in heart rate and blood pressure as well as an prolongation of PR interval. All three of these actions act to reduce the stress on the heart, reducing the symptoms of angina and systemic blood pressure. Propranolol pharmacodynamics can, therefore, be determined by measuring the change observed in any of these three parameters. An electrocardiogram, a relatively noninvasive method for measuring both the PR interval and heart rate, allows for an easy determination of propranolol pharmacodynamics without any undue stress. Using the normal Sprague Dawley rat model, an electrocardiogram allows for a cost effective determination of propranolol potency.

The electrocardiogram is a record of the overall spread of electrical activity through the heart. It is a complex recording of the overall spread of activity

throughout the heart during depolarization and reporalization. An electrocardiogram is not a recording of a single action potential in a single cell at a single point in time. The electrocardiogram has various components, which correlate with specific cardiac events. A normal electrocardiogram has three distinct waveforms; the P wave, which represents atrial depolarization. This is followed by the QRS complex, which represents ventricular depolarization. Finally, the T wave represents ventricular repolarization. The sinoatrial node depolarization does not generate sufficient electrical signal, so no waveform is seen (Figure 1). Therefore, the first recorded wave is the P wave, which occurs when the impulse spreads across the atria. The distance between the P wave and the QRS complex is called the PR interval. This presents the atrioventricular node delay, and is the time required for the current to travel through the atrioventricular node. The atrioventricular node acts to dampen the contraction signal allowing the ventricle time to properly fill. The PR interval, a measure of propranolol potency, is sensitive to any minor changes in calcium channels and β-adrenergic receptor activity. Thus, the PR interval and heart rate are consistent and accurate measures of propranolol activity. [25]

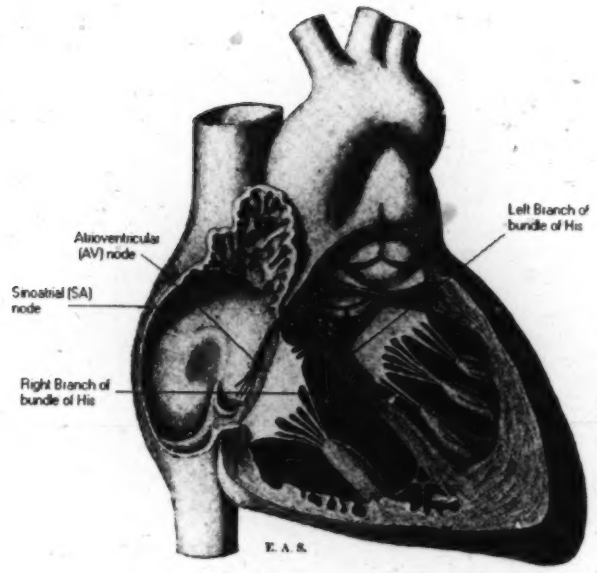

Figure 1 The specialized conductance in the heart. [25]

The Inflammatory Process; The Good, Bad and Useful

The inflammatory process is one of the corner stones of the body's defense mechanism. In its most basic form it acts to defend against foreign entities and expedite their removal from the body. One of regulatory agents, the "police officers" of the body includes cells like the T cells. These and other immunological cells respond to changes in the plasma concentrations of cytokines.

Cytokines are small soluble proteins secreted by various cells throughout the body. Their primary role is a modulator of the immune system. They can be classified into three categories; pro-inflammatory, anti-inflammatory, and growth factors. All the cytokines act in synergy to manage the body's immune response to

anything from the common cold to anaphylaxis. In many cases immunological disorders are seen to be a result of an imbalance in cytokine levels.[26; 27] As well some cytokines demonstrate the ability to be both beneficial and toxic depending on their concentration and site of action.

Interleukin-1 (IL-1) is a stereotypical cytokine that portrays the somewhat conflicting roles of cytokines in the body. During an acute immunological response to simple tissue damage, IL-1, tumor necrosis factor α (TNFα), and interferon γ (IFNγ) are released from damaged tissue and nearby cells. These act to induce the release of several cytokines (IL-1, -2, -6, -8 , TNFα, IFNγ) triggering the " Cytokine cascade".[28]

IL-8 and IFNγ act as chemotactic agents bringing about an infiltration of neutrophils, macrophages and T cells in the site of inflammation. Cytokines act directly to induce increased concentration of nitroic oxide (NO), which at low concentrations can act as free radical scavenger, reducing the effects of free radicals secreted by bacteria and damaged cells. The released cytokines will also act to trigger edema, increase leukocyte adherence to endothelium, and increase production of prostaglandin E_2 (PGE_2), and prostocyclin (PGI). Acutely, therefore, cytokines act to curtail the spread of infection, and increases rate of tissue repair. This is not the case when looking at the role played by cytokines in a chronic inflammatory condition. In situations of chronic inflammation, high concentrations of cytokines will act to trigger bone and cartilage destruction. As well as bringing about an alteration of the hypothalamus/pituitary axis, and contribute to β-islet cells destruction. [26; 27] NO concentrations will proportionally increase in chronic

inflammation, giving rise to cardiac toxicity. High concentrations of NO gives rise to peroxides. Thus, the nature of the cytokine changes from a beneficial controller of immune response, to a direct contributor to body dysfunction.

The dual action cytokines is by no means isolated to individual cytokines, but all cytokines have significantly different effects at different concentrations. Table 1 is a summary of some of the effects attributed to the different cytokines.

Cytokines and their roles

IL-1 and it Potential Therapeutic Use

The use of IL-1 as an immunological modifier is limited primarily by its nature as an "instigator" cytokine. The clinical introduction of IL-1 brought about such significant side effects limiting its therapeutic use. The use of IL-1 was first justified following the observation that IL-1 will up-regulate the expression of class I Major Histocompatibitily Complex on melanoma cells, therefore increasing the recognition and killing of melanoma cells by natural killer cells. As well, studies continue to show that IL-1 significantly modulates the growth of human renal carcinoma cell, and melanoma cells *in vitro*. [29; 30] Phase I clinical trials showed the significant short comings of this observation. Melanoma patients were treated with IL-1α for five days (0.001μg/kg -0.1μg/kg QD, IL-1α was injected subcutaneously into the lesions), the lesions were

Table 1 Cytokine functions

Cytokine Name	Main Cell Source	Category	Function
IL-1	Cells throughout the body	Pro-inflammatory	• Participates in delayed type hypersensitivity reactions. • *CNS*: fever, brain PGE_2 synthesis, increase in ACTH, and Corticosteriods. • *Hematological*: neutrophilia, lymphopenia, bone marrow release inhibitor. • *Hemodynamic effects*: increase nitric oxide synthesis. • *Cytotoxic effects*: tumor cells, β islet cells, and thyrocytes. • *Metabolic effects*: hypozincemia, hypoferremia; decreased cytochrome P-450 enzyme and albumin synthesis; increase in acute-phase proteins, bacterial clearance, and sodium excretion. • increase leukocyte adherence to endothelium. • increase in PDGF, PGE_2, and PGI (procoagulative). • *Proinflammatory effects*: Increase in collagen

			synthesis, cytokine release up regulation, bone resorption is increased, increase in chrondrocyte protease and proteoglycan release.[30-33]
IL-2	TH_1 cells	Pro-inflammatory	• growth and differentiation of T-cells. • Increases production of IFNγ, TNFα, IL-1, CSF and IL-2 receptors. • Growth and polyclonal antibody production. • Macrophage activation, increase in natural killer (NK) cell growth cytotoxicity[30, 33]
IL-3	T-cells (TH_1 and TH_2), NK cells, and mast cells	Growth factors	• is classified as a colony stimulating factor (CFS) • growth and differentiation of myelocytic and erythroid progenitors and magekaryocytes.[30, 33]
IL-4	TH_2 and mast Cells	Anti-inflammatory	• Stimulates TH_2 cells. • Inhibit macrophage activation. (Antagonizes IFHγ) • Increase IgE production. • Increase class II MHC production on macrophages. • Stimulates bone marrow stem

			cells and mast cells. • Induces IL-4, IL-5, and IL-1 receptor antagonist (IL-1Ra). • Reduce production of IL-1, TNF, IL-6, IL-8, and IFHγ. • Generally favors humoral immunity.[30, 33]
IL-5	TH_2 cells	unknown	• Eosinophil growth and differentiation. • Differentiation of B cells and IgA synthesis.[30]
IL-6	Fibroblasts, Macrophages, B-lymphocytes, TH_2 cells, endothelial cells, and keratinocytes.	Pro-inflammatory/ Anti-inflammatory	• B and T cell proliferation and activation. (Increase IL-2 receptor concentration) • Mediates IL-1 effects. • Pyrogenic • Induce hepatic acute phase proteins. • feedback mechanisms for IL-1 and TNF. • Induces CSF[30]
IL-7	Bone marrow, stromal cells, fibroblasts, and placenta	Pro-inflammatory	• Stimulate pre-T and pre-B cells • Increase IL-2 receptor number. • Hematopoiesis and megakaryocyte growth.[30]
IL-8	Monocytes, macrophages, fibroblasts, endothelial cells, keratinocytes, neutrophils, joint fluid, psoriatic tissues, and alveolar fluid.	Pro-inflammatory	• Chemotactic for neutrophils, T cells, and basophils. • Induces neutrophil activation and degranulation. • Augments

			keratinocyte proliferation. Induces local in vivo neutrophil infiltration with secondary edema, and synovitis.[30]
IL-9	T cells	Growth factor	• Supports T cell growth. • protentiates IL-3 mediated mast cell growth. [30]
IL-10	TH_2 cells and B cells	Anti-inflammatory	• Inhibits TH_1 production of IL-2, and IFHγ • Suppresses macrophage production of IL-1, IL-6, and TNFα • Promotes B cell growth and antibody production.[30]
IL-11	Stromal cells	growth factor	• Stimulates Plasmacytoma cell growth • Enhances megakaryocyte growth • supports granulocyte and macrophage colonies • Supplements IL-3 and shortens G_0[30]
IL-12	Monocyte, macrophage, B cells, keratinocytes, and other cells	Pro-inflammatory	• Induction of Th_1 cells, suppression of TH_2 cells. • Induce increased INFγ, TNFα • Promote T and NK cell growth and differentiation. • Enhances anti-tumor activity.[34]
IL-13	T cells	Anti-inflammatory	• Il-4 like activities [33]
IFNα	Leukocytes	Pro-inflammatory/Anti-	• antiviral effects

		inflammatory.	• pyrogenic, anti-proliferative. • Promotes the differentiation of fibroblasts, T cells • enhance the activity of NK cells, B cells • Induce IL-1 and increase TNF receptor numbers. • Induce class I and II MHC.[30]
IFNβ	Fibroblasts, leukocytes, and epithelial cells	Pro-inflammatory/ Anti-inflammatory.	• Presently believed to have same functions as IFNα.
IFNγ	T cells, and NK cells	Pro-inflammatory (immunostimulation)	• Stimulation of T cells, NK cells, and macrophages. • Antiproliferative, antifibrotic • Increases the expression of class II MHC. • Induce; IL-1, IFNα, TNF, CSF, IL-2 receptor. Favors cell-mediated immunity.[30; 33]
IFNτ	Trophectoderm		• Signal for the maintenance of an appropriate milieu for the embryo [35]
TNFα	Cells throughout the body	Pro-inflammatory	• Tumor cells cytotoxicity • Suppresses bone marrow stem cells. • Induces expression of class I MHC • many similar effects as IL-1 and IL-6[30-33]
GM-CSF	T cells, macrophages, endothelial cells, and fibroblasts	Growth factor/ Pro-inflammatory	• Growth and differentiation of granulocyte and

			granulocyte and macrophage progenerators in bone marrow, as well as langerhans cells • induces expression of class II MHC • macrophage activation, cytokines release • Chemotatic for monocytes. [30,33]
G-CSF	Monocytes, fibroblasts, and macrophages	Growth factor/ Pro-inflammatory	• Generates and activates neutrophils. • shortens G_0 of cells cycles. [30,33]
M-CSF	Monocytes, T and B cells, fibroblasts, endothelial cells, and epithelial cells	Growth Factor/ Pro-inflammatory	• generates and activates monocytes. • Stimulates placental trophoblasts. [30,33]
PDGF	Platelets, smooth muscle cells, endothelial cells, and macrophages	Growth Factor	• Induces the expression of class II MHC. • Chemotactic for fibroblasts, monocytes, and neutrophils. • stimulates glial cells, endothelial cells, and osteoclasts to resorb bones. • Triggers vasoconstriction, and promotes wound repair • Matrix and collagen synthesis, as well as induces prostoglandins [30, 33]

TGFβ	Platelets, Placenta, kidney, bone, T and B cells, macrophages	Growth factor Pro-inflammatory/ Anti-inflammatory	• Matrix and collagen synthesis. • Inhibits IL-1, TNF, and IL-1 receptor expression. • Increases the production of IL-1 receptor antagonist (IL-1Ra) • Chemotactic for neutrophils, and fibroblasts • Promotes fibroblast proliferation[30; 33]

biopsied 3 days or 3 weeks later. There was no significant response to the IL-1α therapy. What was seen in all patients was a certain level of IL-1α toxicity which included fever and joint pain.[30; 36] In Phase II trials, the investigators combined IL-1 with indomethacin, in order to reduce side effects, and to potentiate the cytotoxic effects.[30; 33] IL-1 and indomethacin were given to patients with metastatic disease (visceral and non-visceral). In this study a 11% response rate was observed, with one patient showing a significant shrinkage of large liver metastases. [30; 36]

Further studies have looked at the ability to use IL-1 as a hematopoietic agent. Looking at the use of IL-1 for post chemotherapy and bone marrow transplants. In both cases IL-1 would act to trigger a cytokine cascade that results in increased platelet and WBC counts. Several studies have shown that IL-1 would prove useful in such capacity.[30; 36]

In whatever capacity IL-1 is used, a clinically important consideration is its significant toxicity. In many cases researchers have looked to more specific cytokines, that show less toxicity then experienced by many IL-1 treated patients.[30; 36-38]

Anti-IL-1 Therapy in Rheumatoid Arthritis

With the action of IL-1 so ubiquitous in the body, IL-1 was looked upon as a viable therapeutic target for disorders such as rheumatoid arthritis. Although many molecules have been tested an anti-IL-1 has yet to get approval in Canada or the USA. Initial trials although somewhat successful, did not show the complete remissions seen with other anti-cytokine therapies such as anti-TNF. [39; 40]

IL-2 and its Therapeutic Use

Initially discovered in 1976, IL-2 was primarily used as a growth factor for T-cell cultures, presently it is being looked at as a potential adjunct to chemotherapy. IL-2 has shown significant anti-tumor effects *in vivo* when accompanied with lymphokine activated killer cells. [30] The therapeutically administered IL-2 lacks any direct anti-tumor activity, rather acting as a tumor defense system activator. IL-2 triggers the differentiation of T cells in to lymphokine activated killer cells that are cytolytic for various tumor targets. [38] Thus, the exogenous administration of IL-2 acts to up-regulate the immune system, and increase the number of lymphokine activated killer cells. In this way it increases the body tumor defense system. Therapeutically, IL-2 is targeted by cyclosporine in order to prevent transplant rejection.

The exogenous IL-2 administration has proven successful in various cancers; melanoma (17-50 % response), renal cells carcinoma (11- 24 % response), and lymphoma (13-33 % response). In most cases these responses were partial and in no way demonstrated a complete remission of the disease. Trials in which lymphokine activated killer cells were co-administered with IL-2 did not show any significant improvement over IL-2 alone.[37; 38]

As well, researchers have looked at the use of IL-2 in combination with other cytokines, namely the interferons (α and β) for the treatment of cancers. In two separate trials, results have shown that the cytokines act synergistically to increase lymphokine activated killer cell counts and increase therapy response. In one study looking at the response in patients with renal cell carcinoma, there was 27% response. In another study, patients with advanced cancers had varied responses; melanoma 43%, renal cell carcinoma 38%, colorectal cancer 50 % (seen at highest doses). As well, the combined use of cytokines allowed for the use of lower doses and thus reduced toxicity.[37]

Interferons and their therapeutic use

Interferons (IFN) are a group of cytokines that are presently being used therapeutically for their potent antiviral and anti-proliferative effects. In general they are important components of the immune system. They act to trigger the modulation of several genes within target cells, resulting in the combat and destruction of invading viruses, and control of cell proliferation.

In humans their exist two main groups of IFNs, type I IFNs, IFN-α, and IFN-β, these can be secreted from nearly all body cells, but predominantly from leukocytes and fibroblasts. Type II, IFN(γ), is secreted by lymphocytes only .[35]

IFNα/β are presently being used therapeutically for the treatment of several cancers and in the control of chronic hepatitis and multiple sclerosis. The mechanism of action of IFN has been the subject of extensive investigation, due primarily to it's increased use clinically. The antiviral effects of IFNα/β has been believed to be due to the activation of RNase L. RNase L is activated in the presence of IFN's or cytokines, and causes the degradation of cellular and viral RNAs. [35] While its antiviral effects are seemingly well understood, its anti-proliferative effects have yet to be elucidated. It is believed that IFN may have control over several oncogenes, due to homologies between the regulatory sequences of the oncogenes and interferon simulated genes. [35] As well IFN's may act as an immunomodulator, up regulating the bodies natural anti-tumor defense system. IFN's may act utilizing several mechanisms, because IFNα/β continues to demonstrate anti-proliferative effects even in immunodefficient nude mice. [35]

The therapeutic use of IFNα/β has significantly increased since the production of recombinant IFN in the 1980's. Presently IFN is indicated for: hairy cell leukaemia, kaposi's sarcoma in patients with AIDS, chronic active hepatitis B, chronic myelogenous leukaemia , renal cell carcinoma, cutaneous T-cell lymphoma, chronic hepatitis C and multiple sclerosis (Product Monograph for Referon-A). As well, IFN(α) is used experimentally in several other carcinomas. Although indicated for these disease its efficacy is continued to be questioned.

For years IFN α/β was looked upon as an anti-viral agent and thus was initially used primarily in Hepatitis B (HepB). In patients suffering from chronic HepB, IFN therapy has been shown to bring about a complete lose of hepatitis B surface antigen (used as a diagnostic measure of HepB), and normalization of liver function, in approximately 20% of patients, while partial responses are seen in approximately 33-45% of patients. [41] IFNα/β efficacy is limited to individuals having various positive predictors, otherwise there is no significant improvement from control.

IFNα/β has also demonstrated efficacy in other forms of hepatitis, mainly hepatitis C. Clinical trials have shown that partial responses are seen in approximately 50%, and complete remission in 15% of patients undergoing long-term IFNα/β therapy. Presently IFNα/β is also being tested for other forms of hepatitis. Although IFNα/β has limited efficacy in all hepatitis patients, it has become an important agent in fight against the progression of hepatitis.

TNF in Rheumatoid Arthritis

Understanding of the role of TNF in the inflammatory cascade is constantly changing. Researchers continue to state that IL-1 triggers a variety of effects all over the body (Table 1). In studies examining the effects of IL-1 and TNF in *in vitro* and in *in vivo*, the results were virtually identical. Researchers believed the release of one initiated the secretion of the others. Thus it has been called a cytokine network or cascade (Figure 2). It was only with the introduction of anti-TNF α antibody that the picture became clearer. Introduction of anti-TNF antibody into rheumatoid synovial cultures lead to a reduction of IL-1 concentrations. Thus in rheumatoid arthritis TNF-α may be controlling the actions of IL-1 and other cytokines. [42] This does not take away a role for the IL-1 in other inflammatory conditions and its role in homeostasis may still be quite significant. Rather, research has shown that TNF is only one of many cytokines which are activated and present in high concentrations in rheumatoid arthritis (Table 1).[42]

The Effects of Inflammation on Propranolol Pharmacokinetics

Initial observations of changes in propranolol clearance were first reported in patients with elevated erythrocyte sedimentation rate; investigators showed significant increase in propranolol plasma concentration following oral dosing.[5; 6] These observations were later confirmed, by *in vitro* studies [10; 9]using several rat inflammatory models [11; 12; 43; 44] and observed for other highly cleared drugs.[8; 45] The

history of this serendipitous discovery has highlighted the progressive nature of science and paralleled the growth of pharmacokinetics and drug metabolism research.

In one of the first studies looking at the effects of inflammation on drug concentrations, Schneider *et. al.,* looked at the effect of disease such as Crohn's and rheumatoid arthritis on propranolol plasma concentration. Twenty-five rheumatoid arthritis patients were compared to sixteen Crohn's and thirteen control volunteers. An erythrocyte sedimentation rate (ESR) was obtained for each patient at the day of the experiment. Each patient was administered 40 mg propranolol orally and blood samples were taken over six hours. In healthy patients AUC (ng h/ml) was 79.2 ± 13, significantly lower as compared to rheumatoid arthritis patients (ESR < 20, 114.3 ± 19; ESR >20, 570.3 ± 62.1) and Crohn's patients (ESR < 20, 229.2 ± 68; ESR > 20, 544.8 ± 105). The researchers further observed that an elevated erythrocyte sedimentation rate was consistently a predictor of significantly elevated propranolol plasma concentration. The researchers concluded that two possible mechanisms may be responsible for these observations. First propranolol free fraction may be significantly reduced and thus may curtail metabolism, second a modification in liver metabolism may have occurred reducing propranolol clearance. [6] In an endeavor to further elucidate these observations, this research group repeated the experiment using three different β-adrenergic receptor antagonists: propranolol, oxprenolol and metoprolol. These three were picked due to their differing paths of metabolism and pharmacokinetic characteristics. Oxprenolol is predominantly metabolized by conjugation with glucuronic acid while the other

two are not. As well, the three differ in the extent of plasma protein binding, propranolol is 93% bound, oxprenolol 80% and metoprolol only 11%. The three agents were examined in healthy (ESR<20) and in patients with active inflammatory disease (ESR >20). With respect to propranolol the results confirmed previous studies. In patients with an elevated ESR (>20) significantly higher propranolol plasma concentrations were observed. Oxprenolol plasma concentration in patients with elevated ESR also showed a significant increase, almost double. With respect to metoprolol, the observations were quite puzzling, patients with an elevated ESR had almost the same concentration as those with normal ESR. An analysis of these results seemed to point to a relationship between the extent of plasma protein binding and increases in drug concentration. Propranolol with the highest protein binding had the most significant change in concentration, while metoprolol with the lowest plasma protein binding had the least change in plasma concentration. Thus, these results further enforced the theory that an increase in α_1 -acid glycoprotein concentrations and reduction in propranolol free concentrations contributed substantially to the increase in propranolol plasma concentrations. [8] The only weakness with this argument was the lack of statistical power in the metoprolol arm of the study. In the metoprolol arm only six patients were tested in comparison to 38 in the propranolol group. Due to the nature of inflammatory diseases and the fact that metoprolol is a highly extracted drug with a great deal of inter-patient variability, more patients should have been used. The researchers highlighted this point and suggested further work be done. The mechanism(s) responsible for these

observations, modified drug metabolism or protein binding, remained to be conclusively determined. [7]

Increasing evidence was pointing to a significant involvement of inflammation mediators in reduction in hepatic drug metabolism. *In vitro* studies showed that hepatic metabolism and CYP450 content was reduced in inflammed rats. [46] In an *in vitro* study Chindavijak *et. al.,* looked at the effect of inflammation on the metabolism of antipyrine, lidocaine and propranolol in an isolated perfused liver rat model. Using male Wistar rats, inflammation was induced using a hindlimb intramuscular turpentine oil injection 24 h before sacrifice. Rats were divided into three groups, those administered turpentine, saline injected control rats and those pretreated with proadifen, a hepatic enzyme inhibitor. They found that turpentine treatment resulted in significantly reduced clearance of antipyrine; only a trend of reduced clearance was observed in propranolol. Further, proadifen treated rats caused significant reductions in clearance of all three agents tested. Turpentine treatment did result in a significant increase in propranolol half-life. They concluded that the first pass metabolism of propranolol was significantly affected by turpentine treatment, pointing to a role for inflammation modifying liver metabolism. [10] Further hepatocyte studies by this group confirmed significant reduction in propranolol metabolism. [9]

A paper which clearly demonstrated the potential extent of changes that can be triggered by inflammation in drug pharmacokinetics was done by Belpaire *et al.*. Building on the work of Schneider *et al.,* these researchers looked at three drugs with varying extent of protein binding and hepatic extraction. The effect of

turpentine induced inflammation on the pharmacokinetics of three drugs was examined: propranolol, a highly extracted and highly plasma protein bound drug, metoprolol, a highly extracted drug with low plasma protein binding and antipyrine, a low extracted drug. As stated earlier turpentine was injected into hindpaw of animals 24 and 48 h before the experiment. In this case drugs were administered either *i.v.* (propranolol 1 mg/kg , metoprolol 2mg/kg and antipyrine 50 mg/kg) or orally by gastric gavage (propranolol 5mg/kg, metoprolol 10 mg/kg). Serum binding of propranolol and metoprolol was measured to determine changes in protein binding after turpentine administration. [11]

Table 2 The effect of turpentine administration on pharmacokinetics parameters of propranolol, metoprolol and antipyrine. Adapted from Belpaire *et al.* 1989. * Significantly different from control.

	Propranolol		Metoprolol		Antipyrine	
	Controls	Inflammation	Controls	Inflammation	Controls	Inflammation
i.v.	n = 7	n = 6	n =8	n =10	n = 6	n = 7
f_u	0.11 ± 0.008	0.024 ± 0.001 *	0.9 ± 0.01	0.9 ± 0.02	n/a	n/a
$t_{1/2}$ (min)	35 ± 4	24 ± 2	35.6 ± 4	42.9 ± 4	2 ± 0.2	3 ± 0.2 *
V_β (l/kg)	5 ± 0.2	1.4 ± 0.2 *	9 ± 1	10 ± 1	1 ± 0.05	1 ± 0.02
AUC (µg/ml min)	9 ± 1	26 ± 2*	12 ± 2	13 ± 1	n/a	n/a
Cl (ml/kg/min)	109 ± 10	41 ± 3.3*	173 ± 18	153 ± 8	5.5 ± 0.3	4 ± 0.3*
Oral	n = 9	n = 9	n = 7	n =11		
f_u	0.12 ± 0.005	0.025 ± 0.001*	0.9 ± 0.004	0.9 ± 0.01		
AUC (µg/ml min)	4 ± 0.7	83 ± 12	1 ± 0.3	4 ± 0.4*		
Cl (ml/kg/min)	14 ± 2.6	3.0 ± 0.6 *	26 ± 10	3 ± 0.4*		

Turpentine triggered a significant reduction in propranolol, metoprolol and antipyrine clearance after oral administration (Table 2). Propranolol free fraction after turpentine treatment was significantly reduced to 2.5 % from 11 % in control. Researchers concluded that a turpentine treatment resulted in a decrease the *in vivo* metabolism of drugs studied. With respect to changes in propranolol after *i.v.*, they concluded that increased α-acid glycoprotein binding may have contribute to

reduced systemic clearance, but believed other mechanisms must also be involoved. Systemic clearance of highly cleared drugs, as described by the well-stirred model is dependent on blood flow and not enzyme function. Further, changes in systemic clearance can only occur with a change in blood flow or near complete stoppage of liver metabolism. It should be noted that work done in our lab showed no significant change in systemic clearance after *i.v.* administration. [44] After oral administration both metoprolol and propranolol showed significant increase in plasma concentrations, this was attributed to a significant reduction in pre-systemic clearance, which can only occur with a reduction in hepatic metabolic activity. The change in propranolol pharmacokinetics was much more profound after oral dosing as compared to *i.v.*, due to the effect a significant reduction in intrinsic clearance has on pre-systemic clearance of propranolol. These initial studies established the groundwork for a further examination of the processes associated with the changes in drug metabolism.

Mechanisms by which Inflammation Affects Propranolol Metabolism

Initially researchers believed these changes to be associated with increase in α_1 -acid glycoprotein concentrations and reduction in propranolol free concentrations. [47; 48] In one study, researchers examined propranolol pharmacokinetics (*i.v.* and *p.o.* dosing) in rats two days after laparotomy. They found that there was a 2-fold increase in AUC. As well they found that the increase in AUC could be correlated with plasma α_1 -acid glycoprotein. They, therefore, concluded that protein binding must be the main reason for changes in AUC. [49]

Through *in vitro* studies, though, it was determined that the changes in propranolol pre-systemic clearance were due to an inflammation induced reduction in propranolol metabolism.[9] The mechanism(s) responsible for these modifications in cytochrome P450 function has yet to be conclusively determined.

Systemic inflammatory conditions, such as arthritis are associated with widespread increases in circulating inflammatory mediators. [15] Inflammatory mediators such as, nitric oxide (NO) and cytokines (e.g., IL-1, TNF-α and INF-α), as discussed earlier, play a role in almost all biological functions and are believed to play a significant role in the physiological changes observed in patients with inflammatory conditions.[50; 51] With respect to xenobiotic metabolism, inflammatory mediators have been found to act on a variety of cytochrome P450 isozymes.[52]

Inflammatory induction in all its forms has been found to trigger significant reduction in CYP450 activity. Hyperlipidimia, which occurs in more than 50% of Canadians, has been found to reduce the intrinsic clearance of nifedipine. [53] Arteriosclerosis, commonly associated with hyperlipidimia, results in significant elevation in NO and other inflammatory mediators. It is believed that NO generated during the arteriosclerosis process is responsible for the modification of CYP 3A4 and the resulting reduction in nifedipine clearance. [53] These same inflammatory mediators are activated in a variety of situations, from short term or acute elevations associated with child immunization and the common flu to chronic elevation associated with conditions such as rheumatoid arthritis. Thus, the immune systems' regulation of xenobiotic metabolism is not isolated to situations of sever systemic inflammation such as rheumatoid arthritis.

The clinical significance of inflammatory modification on xenobiotic clearance first arose with clinical case reports of modified drug clearance in patients suffering from a variety of systemic infections (e.g., malaria, pneumonia). [52] Latter reports showed that some vaccinations triggered transient increases in theophylline concentrations, but controversy arose due to differing results obtained from different vaccines. [52] As stated earlier, patients suffering from inflammatory diseases such as rheumatoid arthritis, Crohn's disease and ulcerative colitis had a significant reduction in xenobiotic clearance that was correlated to their extent of inflammation, as measured by erythrocyte sedimentation rate.[7; 8] A significant increase in propranolol AUC was exhibited only in patients with an erythrocyte sedimentation rate above 20, therefore, linking a reduction in propranolol clearance with degree of inflammation.

In-order to better understand the mechanisms by which the immune system regulates CYP450, scientists have used a variety of models to mimic the human conditions. One such model is the adjuvant arthritic rat, a systemic inflammatory model triggered by the injection of rats in the tail base with M. *Butyricum* / squalene suspension.[44; 54] In adjuvant arthritic rats propranolol pharmacokinetics was significantly altered, showing a significant increase in AUC accompanied by a significant reduction in the oral clearance (Cl_{oral}) of both propranolol enantiomers.[43; 44; 54] As well, it was observed that the increase in propranolol AUC was associated with disease severity. As the severity of arthritic disease increases so did the increase in AUC and reduction in Cl_{oral}. This reduction is thought to occur primarily in the intrinsic clearance (Cl_{int}) of propranolol, a result of reduced CYP2D6

function. [43; 44; 54] Further when looking at the effect of adjuvant arthritis on propranolol pharmacokinetics, it was observed that an increase in AUC was only seen after oral dosing and not *i.v.*. Normally for drugs undergoing high hepatic extraction, systemic clearance is dependent on hepatic blood flow and thus changes in enzyme activity do not play a role. With respect to oral dosing, though, oral clearance is dependent on enzyme activity and thus a decrease in enzyme activity results in a substantial change in bioavailability and AUC. [44; 54]

The second model, endotoxin model, is an inflammatory model that mimics the effect of a systemic bacterial infection. Inflammation is induced by intra-peritoneal administration of endotoxin 24 h before drug administration. [12] Endotoxins were found to stereoselectively increase AUC and reduce Cl_{int} of verapamil, oxprenolol and propranolol in rats. [12] When the study was replicated in rabbits and dogs, basically the same results were found with a significant increase in AUC and reduction in Cl_{int} for all three drugs studied (verapamil, oxprenolol and propranolol).[45] As a result of experiments performed on these animal models, researchers concluded that an activation of the immune system (i.e. activation of the inflammation cascade) was sufficient to initiate change in CYP 450 activity.

Researchers next examined the role of individual inflammatory mediators on CYP450 activity. The major inflammatory mediators examined were cytokines, an ever-expanding group of immune regulators. They included: TNFβ, IL-1 and INFα-2a. When rats were dosed with INFα2a $5x10^4$ IU S.C. 48 h before verapamil administration, a significant reduction in S-verapamil Cl_{oral} and increased R-verapamil AUC was observed. [55] It was believed that the INFα2a pretreatment acted

to trigger immune system activation. When IL-1β was co-administered with propranolol, there was a significant increase in the concentration of R-propranolol and to a lesser extent S-propranolol, confirming the stereoselective changes seen using the adjuvant arthritis model. [56] The results obtained from single cytokine *in vivo* studies should not be seen as correlative in nature, because the administration of a single cytokine most likely triggers a cascade of responses any one of which may be responsible for the changes seen in CYP450. Thus researchers claiming that any one cytokine is directly associated with CYP450 inactivation, may be inaccurate.[52] However, *in vitro* studies do suggest that a single cytokine may be responsible for CYP450 dysfunction. For example, IL-1 was tested and found to reduce the mRNA content and activity of a variety of CYP450 isozymes (e.g., C211, 1A2, 2E1) [57; 58], as well as reducing the activity of UDP-glucuronosol-transferase and glutathione S-transferase. [59] Similar reductions in CYP450 content were to be evoked by INF α, IL-6, IL-11, IL-8, and IL-2. [52] Interestingly the length of exposure to cytokines was found to influence the results, suggesting cytokine effects are time dependent. Some evidence exists, as well, for the cytokine cocktail, whereby multiple cytokines act synergistically. [52] In a study by Poüs *et al* the effect of IL-1α and TNFβ on CYP 450 content and α_1- acid glycoprotein was examined. Fischer rats were injected with either 1ml IL-1α, TNFβ or both. The resulting observations not only further associated cytokines with reduction in CYP 450 content, but also showed the presence of synergy. IL-1α injection alone triggered a 34 % decrease in CYP 450 content, while TNFβ triggered a 29 % reduction. This in contrast to a over 41% reduction in CYP 450 content when the IL-1α and TNFβ

were combined. Interestingly these results are comparable to a 44% reduction in CYP 450 content after the administration of endotoxin. [60] The potential *in vivo* process of CYP 450 inhibition is most likely initiated and regulated by a verity of cytokines. The fact that a variety of cytokines can trigger CYP450 inactivation (e.g., INF, Il-1) demonstrates that the process by-which cytokines modulate CYP450 has multiple entry points and involves a variety of intermediates.

The Proposed Mechanisms for CYP 450 modification

The modification of CYP 450 activity is thought to occur either by direct interaction or the initiation of a second messenger cascade. When examining the potential mechanisms for CYP 450 inactivation, NO comes to the forefront. NO is generated by nitric oxide synthase (NOS). [52; 61-64] Several isozymes of this enzyme exist, the inducible form is activated by cytokines and other immune modulators.[52; 61-64] One mechanism proposed for the inactivation of CYP 450 is the binding of NO to CYP450 directly. NO can bind to the ferric and ferrous forms of the heme iron, thus preventing the CYP 450 system from accepting electrons and oxidizing the xenobiotic. [52] Some researchers have failed to see reductions in CYP450 mRNA and protein expression when NO donors were used; as well NO-inhibitors did not completely reverse the effect of inflammation on CYP 450 mRNA and protein.[61; 64] Yet others provide clear evidence of the contrary, linking NO directly with CYP450 inactivation. [62; 63] A growing consensus is that multiple mechanisms working at different timelines are acting in conjunction. Cytokine induced NO generation acts quickly to inactivate CYP450 by acting directly on the heme group. Secondly,

cytokines acting on transcription factors reduce mRNA formation, and mRNA half-life.[52] By limiting the time line of an experiment researchers may be only observing the initial NO mediated process, thus concluding that NO is crucially important. Yet other researchers have looked at the long term consequences of inflammation, and conclude that NO does not play a role. In an experiment meant to elucidate the importance of NO in CYP 450 activity modification, Sewer *et al* looked at the down-regulation of CYP 450 mRNAs and protein in mice lacking inducible nitric oxide synthase.[65] Endotoxin was used to initiate a systemic inflammatory reaction in both control and inducible nitric oxide synthase knockout mice. At 6 and 24 h after injection of endotoxin animals were sacrificed. Knockout mice treated with endotoxin for 6 or 24 h showed no increase in plasma NO concentration. Yet, a significant reduction in CYP 2C29, CYP 3A11 mRNAs and protein content was observed in both strains of mice. An examination of the hydroxylase activity (i.e., the rate of formation of a variety of testosterone metabolites) showed that in knockout mice no reduction in activity was observed. These results clearly show that NO is not involved in the down-regulation of CYP 450 mRNAs or protein. However, a role for NO seems to exist in the inhibition of catalytic activity.[65] The reality is that the actions of inflammation are dynamic and are affected by several physiological factors and should be examined with this in mind.

Although these studies have shed light on the mechanisms by which inflammation modifies CYP450 function, they fail to address the therapeutic consequences of these drastic increases in plasma xenobiotic concentrations. What are the clinical consequences to patients with chronic inflammatory conditions, such

as arthritis? Do increases in drug concentration, such as propranolol, trigger toxicity?

Effect of inflammation on Propranolol pharmacodynamics

Propranolol is a nonspecific β-adrenergic receptor antagonist, which acts to block the activity of β-adrenergic receptor agonists such as epinephrine and norepinephrine. The β-adrenergic receptors form the backbone of the sympathetic system and are the site of action for a variety of neurotransmitters, hormones and therapeutic agents. The β-adrenergic receptors are characterized as G-protein coupled receptors, with the G-protein acting as an intermediate agent between the receptor and a large number of diverse effectors (e.g., adenylate cyclase, phospholipase, and a variety of ion channels). Upon receptor activation, an association with the G-protein complex occurs and a cascade of effector activity is triggered. The G-protein is composed of three subunits, the α, β and γ; in the basal state GDP is bound to the GTPase catalytic site of G_α. Upon association with the receptor, GTP displaces GDP and the $G_{\alpha\beta\gamma}$ is disassociated into G_α and $G_{\beta\gamma}$ subunits. The G_α and $G_{\beta\gamma}$ subunits proceed to either positively activate or inhibit a number of cellular activities. [66; 67]

The β- adrenergic receptors system is composed of a variety of different β-receptors, which can interact with a variety of G-protein complex isoforms. Currently three β- adrenergic receptors subtypes have been cloned and pharmacologically characterized (i.e., β_1, β_2 and β_3), a fourth subtype β_4 may also

exist but is not well characterized. These receptors have a variety of effects depending on which G-protein complex they associate with. This inherent regulatory system is ideal for modification. Evidence for cytokines acting as β-adrenergic receptors modulators is growing; with numerous clinical and *in vitro* studies linking modification in β- adrenergic receptors with the presence of cytokines.

The Effect of Inflammation on the β-Adrenergic System

There are several therapeutic and research areas providing evidence for the involvement of inflammation in disease pathology and etiology. Two note worthy areas are asthma and cardiovascular disease. I will be looking at these areas in detail and try to summarize the role of inflammation in modulation of the β-adrenergic system.

Asthma:

The importance of inflammatory modulators in β-adrenergic receptor function seems to have come to light when examining refractory asthma patients (i.e., uncontrolled active inflammation in the lungs). Refractory asthma patients have demonstrated reduced or limited response to β_2-agonist. The primary belief was that reductions in β-adrenergic receptor effectiveness were due to repeated use. However, desensitization only occurred in refractory patients with active uncontrolled asthma. Thus the question arose, what is responsible for receptor dysfunction? Is it repeated use of agonist or active uncontrolled asthmatic disease?

In several clinical studies, the use of inhaled steroids (primary anti-inflammatory used in asthma), was associated with increased β_2-agonist effectiveness. [68; 69] Thus, with reduction in the inflammation (asthma control) you get increased response to β_2-agonist.

The direct association of inflammatory agents and reduced β-adrenergic receptor function was further established with *in vitro* studies. Early studies investigated the role of prostaglandins and leukotriens in human airway tissue desensitization by examining receptor number and affinity using radioligand-binding studies. One study showed a reduction in receptor number and affinity in the presence of prostaglandins. [70] As research progressed a focus was directed on the mechanism(s) by which these changes in receptor response may be occurring. One potential area was the G-protein coupled second messenger system, this cascade may be modified by inflammatory mediators such as cytokines. The studies, initially, examined the basic response of β_2 adrenergic receptor response to isoproterenol, in the presence and absence of various cytokines (i.e., IL-2β, TNF-α and IL-2). The results were surprisingly consistent with a significant reduction in isoproterenol mediated responses in the presence of pro-inflammatory cytokines (i.e., IL-2β and TNF-α). [71-73] A more detailed examination of the cytokine effects on the second messenger system revealed a general pattern. Cytokine treatment triggered a modification in the cellular protein levels of G-proteins, with G_i levels increasing in the presence of reduced G_s; thus a shift to greater β adrenergic receptor coupling to G_i. These observations were consistent with the reduced isoproterenol induced cAMP production in the presence of cytokines. [71-73] Controversy arose,

though, when looking at the receptor itself, its number and affinity. Some researchers showed a biphasic change in receptor in the presence of IL-1β (i.e., an initial increase followed by a reduction).[73] While, others showed no change in either receptor number or affinity[71], yet another group demonstrated significant reduction in receptor density.[72] These observations, although contradictory, highlight the nature of cytokines in general. The cytokine effect is very time and concentration dependent, and minor changes in protocol bring about a significantly different response.

Although not completely understood, the link between inflammation and β_2-adrenergic receptor function in the airway tissue is well entrenched. This has allowed for a new direction in patient therapy with greater emphasis being placed on regular and early steroid therapy.

Cardiac Disease:

In recent years, a paradigm shift in our understanding of cardiac disorder etiology has occurred. Increasing emphases has been given to reduction in the extent and degree of ischemia with reperfusion therapy. Ischemia is an initiator of the inflammatory cascade, with the release of free radicals triggering cell damage, activating immune cells and cytokine release. It is believed that an ischemia initiated inflammatory cascade contributes significantly to modification in cardiac physiology. The studies examining cardiac β-adrenergic receptor dysfunction have focused on a variety of different cardiac conditions.[66; 67] Initial studies, were clinical or observational in nature, linking cytokine increase with cardiac

dysfunction and patient survival. [74-79] The role of inflammatory processes in the etiology cardiac disease came to light only when researchers started looking at the potential for cytokines to directly affect cardiac receptors and ion channels. In the endotoxin *in vivo* model, which induces sepsis and is associated with a significant increase in circulating cytokines, significant cardiac dysfunction is triggered. [80; 81] Examination of β-adrenergic receptors through radiolabelled binding studies, reveals modifications in receptor densities and reduced response to agonist (i.e., reduced formation of cAMP in presence of isoproterenol). This was also accompanied with increased rate of β-adrenergic receptor phosphorylation. [81]

In *in vitro* studies examining the role of individual cytokines revealed similar results. When IL-1β was introduced to normally functional cardiac myocytes, a significant decrease in cAMP formation in response to isoproterenol, was observed; as well as modification in calcium current. [82-85] Studies on the second messenger system showed signs of increased receptor phosphorylation and reduced G_s concentrations. IL-1 introduction is thought to have triggered these changes. To some extent similar results were found when TNF-α was used, with a significant reduction in cardiac β-adrenergic receptor response observed. [86]

In an effort to further link cytokines to the β-adrenergic receptor modifications seen in cardiac disease and sepsis, anti-cytokine antibodies were used. When anti-TNFα was introduced to rats with endotoxin induced sepsis, some of the β-adrenergic receptor dysfunction was reversed, this included a normalization of cAMP formation. Most notably though, anti TNF therapy failed to reverse the

modification in β-adrenergic receptor density. [87] In a recent study with advanced heart failure patients were treated with a synthetic TNF soluble receptor (Etanercept, ENBREL®), which acts to bind and inactivate circulating TNF. The administration of etanercept resulted in dose dependent improvement in left-ventricular ejection fraction and left-ventricular remodeling as well as patient functional status. [88] All these studies provide growing evidence of a role for cytokines in triggering cardiac dysfunction. In spite of this extensive level of data, limited information exists on the mechanisms by which cytokines trigger these changes.

Potential Mechanisms of Action for Inflammatory Mediators

The role of cytokines and inflammatory mediators in β-receptor dysfunction is entering mainstream clinical practice. The aim of therapy is being focused on reducing inflammatory mediators in order to normalize function. The questions yet to be addressed is the pathway(s) or mechanism(s) by which these inflammatory mediators bring about modification in β-adrenergic receptor function. There are several theories by which this occurs. They include direct or indirect cytokine action on receptors. Further, there is also controversy on the role of NO in these modification: is NO the main agent of action or only a secondary instigator of this action? In the following sections these points will be addressed and summarized.

The Direct Theory:

Consensus has been reached when addressing the mechanism by which cytokine mediated receptor desensitization occurs; *this is not a simple mechanism.* One possible mechanism is receptor sequestration. The sequestration process is

triggered by β-adrenergic receptor phosphorylation, arrestins binding follows, allowing for the internalization of the receptor into clarthin-coated vesicles. [66; 67; 90] An examination of some *in vitro* radiolabelled ligand binding studies shows no significant changes in receptor number or affinity [70; 71; 83; 85; 91; 92]; suggestive of involvement of other mechanisms.

In many papers looking at inflammation mediated β-adrenergic receptor changes, researchers have cited a significant change in the G_s/G_i ratios. Increase in G_i concentration requires transcriptional up-regulation and a reduction in G_s formation. Increased concentrations of G_i can bring about several mechanisms of receptor deactivation. Either the full G_i-protein complex or individual $G_i\beta\gamma$ subunits can act to trigger reduced cAMP formation, hence reduced receptor function. [70; 71; 83; 85; 91; 92] Not withstanding the greater potential for β-adrenergic receptors to bind to G_i then G_s. The inflammation-induced reduction in cAMP generation in the presence of agonist can also be produced by an increased cAMP breakdown. This is thought to occur via cytokine-induced up-regulation of phoshodiesterase-4 activity acting to quickly increase cAMP metabolism and thus reduce β-adrenergic receptor activity [89] Finally researchers have noted a reduction in the calcium current generated in contraction, thus a modification in the excitation-contraction coupling. This is thought to occur due to a modification in the calcium-induced-calcium release threshold and L-type calcium channel. [93; 94]

The Indirect Theory:

The desensitization of receptors has long been believed to be a physiological response to the repeated exposure to agonists. In a variety of cardiac diseases catecholamines are elevated, the clinical importance lies in addressing the cause for these increases. [66; 67; 90] With growing evidence, catecholamine elevations are becoming part of the disease symptomology and etiology has been focused on the role of the immune system. *In vivo* studies looking at the effects of endotoxin or IL-1β have shown an increase in circulating catecholamine concentrations upon introduction of the inflammatory agents.[95] It is possible that a cytokine-mediated increase in catecholamines can trigger receptor desensitization via sequestration or other process such as receptor phosphorylation.

The Nitric Oxide theory:

In various inflammatory conditions, in addition to increased expression of cytokines, there are a significant increase in circulating NO concentrations, indicating a link between cytokine and NO concentrations. Early studies quickly linked increased NO concentrations to cytokine-mediated processes. [96] NO is produced from L-arginine by the actions of nitric oxide synthase (NOS). [52; 61-64] Three basic forms of nitric oxide synthase have been isolated, two constitutive forms called cNOS and they are localized in neural tissue (Type I) and endothelium Type(II). The third form is inducible and is called iNOS. It is this form that is activated by cytokines, such as IL-1β. Once activated an increase in NO levels occurs in several tissues and throughout the body. A high concentration of NO acts

to increase the intracellular cGMP, which in turn acts to reduces calcium ionic current in the cell. The reduction in intracellular calcium results in diminished muscle activity. As well, large concentrations of NO trigger the formation of oxygen free radicals, which are responsible for triggering free radical mediated cellular damage. The majority of these findings have been experimentally proven using two agents; N^G-monomethyl-L-arginine, a NOS blocking agent, can cause reduced NO formation and L-arginine a NOS substrate, causes increase NO formation. In *in vitro* experiments pre-incubation with N^G-monomethyl-L-arginine resulted in reversal of IL-1β effects. On the other hand pre-incubation with L-arginine resulted in augmentation of IL-1β effects.[84; 94; 97] Yet another study using cardiac myocytes and multiple cytokines suggested that N^G-monomethyl-L-arginine can only reverse the affects of IL-6, but not TNF-α.[93] Therefore, demonstrating the presence of an NO-independent mechanism.

NO-independent Theory:

For a long time scientists have recognized the existence of sphingolipids in the cell membrane. Recently, cytokines have been implicated in their conversion into second messengers such as ceramide and sphingosine. The sphingomyelinase pathway has been recognized as a major second messenger pathway for TNF. TNF activated sphingomyelinase acts on sphingomylein to give rise to ceramide, which can be subsequently converted to sphinogsine.[98-102] Sphingosine can depress cardiac function by acting on the intra-cellular and extra-cellular calcium.[103] It is also believed that sphingosine can inhibit calcium induced-calcium-release,

increasing the threshold for intracellular calcium-release. As well, sphingosine can affect L-type-channel conductance, reducing extra-cellular calcium current. In several studies, the effects of TNF on cardiac function was reversed with the introduction of a ceremidase inhibitor, N-oleoythanolamine. These results demonstrate the presence of a NO independent pathway evolving sphingosine and TNF. [86; 93] The majority of studies using TNF and NO demonstrate a significant reduction in cardiac function in the presence of TNF. In one study though, researchers found that TNF may have cardio-stimulatory functions. In β-adrenergicly blocked conscious rats, a one hour infusion of TNF triggered a biphasic response. Within the first 15 min, left ventricular contractile performance increases up to 44%, this was followed by a gradual and sustained reduction one hour after the completion of the infusion. [95] This study does again show the time dependent nature of cytokine effects and may demonstrate the presence of multiple site of action for TNF and its role in body homeostasis.

Treatment Options for Inflammation

Inflammation is an easily initiated process but difficult to control. A variety of chronic conditions are associated with an uncontrolled inflammatory process. Rheumatoid arthritis, with a prevalence of 1%, is one of the most commonly occurring systemic afflictions with no cure in sight. Characterized by chronic inflammation in the synovium of joints, it results in cartilage degeneration and erosion of juxta-articular bone. As well, it is distinguishable by an elevation of the pro-inflammatory cytokines systemically and in the affected joints. [42] Traditionally,

patients with rheumatoid arthritis or other inflammatory conditions have been primarily treated with corticosteroids or NSAIDs for the reduction in inflammation. Unfortunately, however, these agents have long-term side effects. Disease modifying agents such as hydroxychlroquine, gold salts and methotrexate were used to reduce progression of the disease. Recent changes in clinical practice have focused on early prevention of disease progression, thus resulting in the increased use of modifying agents.

New Approach to Arthritis Treatment

A new approach to arthritis treatment is to directly inhibit the activity of inflammatory mediators (i.e., IL-1and TNF). The most clinically successful approach has been to bind circulating TNF-α by administering with TNF-α anti-bodies (infliximab, Remicade, Schering Canada Inc. Pointe Claire, Quebec, Canada) or synthetically produced soluble receptors (etanercept, Enbrel, Immunex).[104-108] The use of anti-TNF agents arose from evidence pointing to TNF as one of the first cytokines to be elevated in the early initiation of inflammation and the instigator of the inflammatory process, although evidence exists that a variety of cytokines (e.g., IL-1, INF- α) if exogenously introduced trigger the entire inflammatory process. The first anti-TNF agent to be tested was infliximab. In a Phase I/II open trial infliximab was tested in refractory rheumatoid arthritis patients. A high dose was initially used (20 mg/kg over 2 weeks). The results were notable, with patients reporting alleviation of symptoms such as pain, morning stiffness, tiredness, and lethargy within hours of therapy. A reduction in the number of swollen joints and

tender joints was observed by 2 to 4 weeks. [42] While all patients tested responded, the effects were temporary, with the response lasting only 8-22 weeks. In the placebo controlled clinical trials, two doses of infliximab (1- 10 mg/kg) were used. It was found that the low dose (1 mg/kg) response rate was 44%, while a high dose (10 mg/kg) of infliximab resulted in a 79% response rate. The degree of improvement was high, 60-70% reduction in disease activity measures such as tender or swollen joints and C-reactive protein (a general indicator of inflammation). As well, it was found that with low dose therapy the median duration of response was 3 weeks, as compared to 8 weeks achieved with high dose therapy. Infliximab was also found to affect downstream cytokine release. [42] For example, within a day of infliximab therapy, serum IL-6 fell to normal levels, and C-reactive protein levels were normalized in a few days. As well there is also evidence for reduction in IL-1 and IL-8. [42] Although, notably quite significant, most of these responses had limited duration. Thus studies were designed to determine if long-term anti-TNF therapy was safe and effective. In the presence of low dose weekly methotrexate injections, it was found that repeated high does infliximab were safe and showed a sustained response. [109-111] Although these new therapies are quite effective, due to their cost mainstream use is restricted. The front line agents for regular analgesic and anti-inflammatory control continue to be NSAIDs and disease modifying agents.

The Role of NSAIDs in Rheumatoid Arthritis Therapy

It is estimated that 27% of Albertans over the age of 65 receive at least one prescription for an NSAID every six months.[1] NSAIDs act to inhibit the actions of cyclooxygenase (COX), thus preventing the formations of prostaglandins from arachidonic acid . [112; 113] COX has been found to consist of two isoforms, COX-1 and COX-2. The two isoforms have differing physiological and pathological functions. COX-1 activity is constitutive, present and active in nearly all cell types at a constant level. With respect to COX-2, however it is normally absent from cells and its formation is regulated by external stimuli such as cytokines. [113] First generation NSAIDs such as flurbiprofen act to inhibit both COX isoforms.

The inhibition of the constitutively active COX-1 by first generation NSAIDs triggers a disruption in homeostatic regulation. Inhibition of COX-1 results in a reduction of prostaglandins required for homeostasis. This triggers several clinically significant side effects including: gastrointestinal (GI) toxicity (i.e., ulceration and inflammation) and exacerbated kidney dysfunction. [112; 113] The reduction in prostaglandin formation at the GI track results in a destruction of cell lining integrity, thus increasing the incidence of GI ulceration and inflammation.

Gastrointestinal Toxicity and NSAID Therapy

Patients with osteoarthritis or rheumatoid arthritis use NSAIDs on a daily basis just to maintain mobility and quality of life. Unfortunately as much as 65% of patients show signs of NSAID induced GI toxicity; from minor sub-clinical changes in intestinal permeability to intestinal lesions and ileal dysfunction, after as little as

six months therapy. [115; 116] In a retrospective study from Finland looking at the causes of death in 1666 rheumatoid arthritis patients, it was found that 47 died from the adverse effects of anti-rheumatic drugs. Among these adverse effects 30 were attributable to NSAIDs. [117] The mechanism by which NSAIDs trigger significant side effects has been associated with reduced COX-1 activity, thus increasing intestinal cell turnover and causing ulceration and perforation. In an endeavor to reduce or eliminate GI specific side effects, several investigators tested agents that may reduce or prevent these side effects. The two agents that were extensively tested were misoprostol and metronidazole. In initial rat studies it was found that pre-administration of metronidazole 12 h and 1 h before flurbiprofen administration prevented change in intestinal permeability, and thus eventual ulcer formation. [118] In a crossover placebo controlled clinical trial patients were given indomethacin then placebo, misoprostol or metronidazole. Patients co-administered with metronidazole and indomethacin had no change in GI permeability, while those given misoprostol still showed significant change in permeability.[115] Further studies have shown that although misoprostol does not prevent change in GI permeability it is useful in reduction in GI side effects and treatment.[116] Clinical use of metronidazole was not pursued as it had significant side effects.

Another approach to the prevention of GI toxicity involved the use of NSAIDs that were more specific to COX-2 rather then COX-1. In several studies it has been found that with increased COX-2 specificity there is reduced potential tendency for GI toxicity.[117; 119; 120] With this in mind drugs such as celecoxib and rofecoxib were developed. Several clinical trials and continued market surveillance

have shown that both celecoxib and rofecoxib are safer then traditional NSAIDs, with respect to GI toxicity. Although they have reduced rates of GI toxicity, it should be noted that they are not completely free of GI side effects and caution is still advised with patients with a history of GI ulcers.[114; 121-123]

Renal Function and NSAID Therapy

The effect of NSAIDs on kidney function and the clinical consequences of these actions have been always controversial. With respect to kidney function, the inhibition in COX-1 triggers reduced sodium and water secretion (Figure 8). This causes increased water retention and potentially clinical side effects in those with existing renal or cardiovascular pathology. Second generation NSAIDs such as celecoxib and rofecoxib, which are relatively specific COX-2 antagonists, are believed to lack some of these side effects. [114; 121] Studies have shown that COX-2 is present in the renal vasculature, cortical macula densa, and the medullary interstitial cells of the kidney. Most interesting COX-2 content in these areas increases with age. In contrast COX-1 is found in the vasculature, the collecting ducts and the loops of henle. The presence of both forms in the vasculature posses the question, which is more important? [114] Recent clinical studies, though, have highlighted the possibility for a role for COX-2 in normal kidney homeostasis. Celecoxib and rofecoxib have been found to affect kidney function in individuals with compromised function and secondary complications (e.g., hypertension, congestive heart failure). [124; 125] Studies conducted in an elderly population currently on antihypertensives showed that celecoxib and rofecoxib can trigger a 2-4 mmHg

increase in blood pressure, thus nullifying the effect of many antihypertensives. In a head to head comparison of celecoxib and rofecoxib, eight hundred and ten patients received either rofecoxib or celecoxib. Patients included for this six week trial were 65 years or older, had controlled hypertension, were diagnosed with osteoarthritis and would benefit from NSAID therapy. Clinical and laboratory assessments were performed at baseline and after 1, 2 and 6 weeks. Nearly twice as many rofecoxib treated patients developed edema as compared to celecoxib. At the 6 week point mean systolic blood pressure was increased by 2.5 mmHg in the rofecoxib group. [125] This is in contrast to literature that states COX-2 antagonists only cause transient sodium retention in healthy individuals. In normal subjects 59-80 years of age administration of 50 mg of rofecoxib caused a 20% reduction in urinary sodium excretion, but caused no decline in glomerular filtration rate or any detectible edema or hypertension. [114; 126] Thus many scientists continue to believe that glomerular function, even in normal elderly, does not depend on renal COX-2 function.[127] Thus the role of COX-2 in kidney function has yet to be conclusively determined. What cannot be refuted is that COX-2 specific agents such as celecoxib and rofecoxib, like their traditional NSAID cousins, pose a clinical threat to those with compromised renal and cardiovascular function.

Role of NSAID Therapy in Reduced Blood Pressure Control

Blood pressure control in hypertensive patients is significantly reduced by NSAID therapy, due to reduction in kidney function, resulting in increased water retention. [14; 129-134] Traditional NSAIDs, and to a limited extent second generation

NSAIDs, act to reduce prostaglandin formation in the kidney which results in an increase in sodium and water retention. This is transient and can result in a 0.5 – 3.7 mmHg increase in blood pressure. [14] A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure examined fifty four studies. The mean subject age was 46 years, of the 1324 participants 92% were hypertensive. The effects of NSAIDs on blood pressure were found solely in hypertensive subjects. Patients administered naproxen and indomethacin had an average increase in mean arterial pressure of 3.7 ± 2.0 and 3.6 ± 1.1 mmHg respectively. [14] In yet another meta-analysis investigators selected randomized, placebo controlled trials comparing one or more NSAIDs. Again they found a significant increase in mean arterial pressure (5 mmHg) with the use of piroxicam in hypertensive patients. They also concluded that elderly hypertensive patients taking NSAIDs had a greater likelihood of having systolic blood pressure greater the 140 mmHg, thus demonstrating uncontrolled hypertension. They also concluded that NSAID use may be an independent factor for hypertension in non-institutionalized elderly patients. [133] Increased blood pressure in the elderly may have substantial clinical consequences, notably heart failure. In a Swedish study looking at hospital utilization of patients diagnosed with heart failure, found that 27 % of them utilized NSAIDs. As well, the use of NSAID correlated with increased hospitalization utilization in general. [135] In a similar study conducted in New South Wales, UK, it was found that NSAIDs contributed to approximately 19% of hospital congestive heart failure admissions. [136] What is surprising in all these studies is the fact that all these patients were on antihypertensive therapy and presumably had stable and

controlled hypertension. Studies have concluded that NSAIDs must be acting directly on patient antihypertensive therapy. [132; 134; 129; 131]

Chapter II: Objectives and Rational

The Consequences Of Inflammatory Modifications

Inflammation brings about significant modifications throughout the body. Inflammation induced increase in cytokines and NO, which act to significantly reduce pre-systemic propranolol metabolism, triggering a significant increase in propranolol plasma concentrations. Cytokines also have been found to act directly on calcium currents, and β-adrenergic receptor function. What are the clinical consequences of these modifications? "What effect does receptor modification have on the efficacy of medications administered to patients with inflammatory conditions?" The primary objective of this study was to determine the consequences of increased inflammatory mediators on the pharmacokinetics and pharmacodynamics of propranolol in an *in vivo* arthritis model. By using a validated chronic inflammatory model, adjuvant arthritis, we tried to determine if modifications in propranolol pharmacokinetics may lead to modifications in propranolol pharmacodynamics.

Hypothesis

Inflammation will give rise to a significant elavation in propranolol pharmacokinetics, accompanied with a modification β-adrenergic receptor function. This results in no change in propranolol potency.

What are the Treatment Options?

Several studies have recently shown that anti-TNF therapy can reduce symptoms of rheumatoid arthritis.[42; 109; 110] Rat studies have shown anti-TNF therapy significantly improves symptoms in the adjuvant arthritis model.[137] As well, anti-TNF therapy has been found to increase heart function in patients with heart failure. Thus it is possible that reducing circulating TNF concentration may normalize propranolol metabolism. What would its effect be on propranolol pharmacodynamics? Thus at this point an exploratory study was designed to examine these questions. The objective was to determine if anti-cytokine therapy (i.e., anti-TNF antibodies) could reverse the effects of inflammatory mediators on propranolol pharmacodynamics and pharmacokinetics. This would allow us to determine if TNF has significant links to the effects of inflammation on propranolol pharmacokinetics or pharmacodynamics.

Hypothesis

Anti-TNF therapy will bring to normal propranolol pharmacokinetic and pharmacodynamic parameters.

NSAID Effects on Antihypertensive Agents

NSAID therapy is associated with a high rate of GI toxicity in the form of ulceration and inflammation.[138; 139] Gastrointestinal ulceration has been found to

bring about significant elevation in inflammatory mediators.[140; 141] The effects of NSAIDs on cytokine levels have been studied thoroughly and most results show some reduction in cytokine levels. [33; 74; 142-152] Yet a closer examination reveals that NSAID therapy significantly increases baseline TNF-α [146; 153-155], IL-2 and INF-γ [156]. In one study, indomethacin therapy resulted in a significant increase in circulating TNF levels, associated with GI ulcer formation. The elevation in inflammatory mediators may act to modify β-adrenergic receptor function. This may be a mechanism by which NSAIDs affects anti-hypertensive effectiveness. Our aim was to determine if NSAID therapy has an effect on propranolol potency and/or baseline cardiac indices in Sprague Dawley rats and if this is linked to GI toxicity. This would allow us to determine if NSAID induced GI inflammation may contribute to reduced propranolol potency.

Hypothesis

Traditional NSAID therapy will cause significat changes in cardiac baseline indices (PR interval, heart rate and blood pressure), as well as reduce propranolol potency.

Chapter III: Experimental Procedures

Materials:

Racemic propranolol was purchased from Sigma-Aldrich (Oakville, Ontario Canada). Bupranolol was obtainded from Logeais (Issy-Les-Molineaux, France). S-(+)-1- (-- 1-naphthly) ethyl isocynate was purchased from Sigma-Aldrich (Oakville, Ontario Canada). *Mycobacterium Butyricum* was purchased from Difco Lab. (Detroit, Michigan, USA). Ketoprofen, flurbiprofen, indomethacin were purchased from Sigma-Aldrich. Celecoxib and metronidazole was purchased from a near by pharmacy. Infliximab was obtained from Schering Canada Inc. (Pointe Claire, Quebec, Canada). Isoflurane was obtained from Abbott Laboratories (Montreal, Canada). HPLC Grade hexane was obtained from EM Science (Grelbsitein, N.J., USA) Chloroform, diethyl ether and methanol (all HPLC grade) were obtained from Caledon (Georgetown, Ont., Canada).

Instrumentation and equipment:

High-Performance Liquid Chromatography:

The HPLC apparatus (Waters, Mississauga, Canada) consisted of a Model 590 pump, Waters 740 Scanning Fluorescence detector, a 712 Wisp auto-sampler, and a Whatman Partisil 5 (Clkifton, NJ, USA) 25 cm stainless-steel silica column. The analytical column was operated at ambient temperature. The recorder-integrator was a Model 3390 A(Hewlett-Packard, Palo Alto, CA, USA). Vortex used was a

Vortex Genie 2 mixer (Fisher Scientific Industries, Springfield, MA, USA). Savant speed Vac concentrator-evaporator (Emerston Instruments, Scarborough, Ontario, Canada) was used for drying of samples. Mobile phase was filtered using a Corning Mega-Pure SA-70 System (Corning, NY, USA)

Electrocardiogram recording and Analysis:

Teflon coated electrodes were purchased from Biomed Wire (Crooner Wire, Chatsworth, California, USA). Signal was amplified using a Honeywell ECG amplifier then digitalized, filtered and analyzed using Acknowledge 3.01 data acquisition system (BIOPAC Systems Inc. Santa Barbara, California, USA).

Ethics approval:

All animal experimentation protocols were reviewed and approved by the University of Alberta Animal Care ethics Committee.

Animals:

Male rats (250-350 g) were of the Sprague-Dawley strain and raised in house by the University of Alberta Animal Colony Facility. Animals had an average age of approximately 6 months. Animals were housed at ambient temperature and humidity in individual cages. They were fed a standard rat chow and allowed free assess to food and water for the duration of the experiment unless otherwise stated.

Propranolol HPLC assay:

Propranolol enantiomers were quantified using the following assay from Piquette-Miller and Jamali (1993). [44] To 0.1 mL of plasma, 50 µL of internal standard (bupranolol, 2.5 µg/mL) and 250 µL of 0.2 NaOH were added. The mixture was extracted with 5 mL of diethyl ether, vortex-mixed for 30 s, and centrifuged at 1800 g for 5 min. The organic layer was transferred to a clean tube and evaporated to dryness using a Savant speed Vac concentrator-evaporator. The residue was then derivatized with 185 µL of 0.02% S-(+)-1- (-1-naphthly) ethyl isocynate in hexane and vortex-mixed for 60 s. Aliquots of 150 µL were injected into the HPLC. A mobile phase of hexane:chloroform:methanol (75:25:0.4), pumped at 2mL/min, was used. The fluorescenee detector was set at 225 and 330 for excitation and emission, respectively.[44] Calibration curves were linear over the concentration range of 250-50000 µg/L of propranolol enantiomers (correlation coefficient r>0.99, coefficient of variation less then 10%). The limit of detection was 50 µg/L.

Induction and Assessment of Adjuvant Arthritis

In order to induce adjuvant arthritis, Sprague-Dawley rats were injected in the tail base with 0.5 ml of a *M. Butyricum* in a squalene suspension. Rats were then monitored for two weeks at the end of which their arthritic index was determined. [44] The arthritic index is a quantification of joint swelling and dysfunction in the rat. Both hind and forepaws were assessed. Each hind paw was given a score of 0 to 4 in the following basis: 0 = no joint involvement, 1 = a single

joint, 2 = more than one joint involved, 3 = the involvement of several joints and moderate swelling of ankle, 4 = the involvement of several joints and sever swelling of the ankle. Each forepaw was given a score of 0 to 3 in the following basis: 0 = no joint involvement, 1 = a single joint, 2 = more than one joint involved and/or wrist, 3 = involvement of wrist and several joints with moderate-to-severe swelling. The maximum possible arthritic index score is 14.

Propranolol Dose effect curve:

Sprague-Dawley rats were randomly divided into, control non-arthritic (n= 17) and arthritic groups (n= 14). When adjuvant arthritis was evident, both groups underwent the experimental protocol. Anesthesia was initiated with ether and maintained with isoflurane. ECG electrodes, teflon coated wire, were placed in the dermis at the right and left axial regions and near the xyophoid process. Animals were allowed to recover for 2 h, and a baseline ECG was obtained. In has been reported that the effect of anesthesia used in this experiment on ECG diminishes in 2 h. [157] Single doses of 3, 5, 10, 20 and 30 mg/kg racemic propranolol were administered orally 15 min after establishment of the baseline. ECG was recorded at 5, 10, 15, 30, 60, 120, 180, 240 and 360 min post dose.

For further studies we chose single doses of 25 mg/kg since the slope of the dose-PR interval prolongation curve appeared rather steep within the 20-30 mg/kg range.

Simultaneous propranolol pharmacokinetics and pharmacodynamics monitoring:

Sprague-Dawley rats were randomly divided into, control non-arthritic (n= 4) and arthritic groups (n= 4). When adjuvant arthritis was evident, both groups underwent an experimental protocol similar to that explained under 'Propranolol dose effect curve' except that, for the pharmacokinetics study, the day before propranolol administration a silastic catheter was inserted into the right jugular vein. On the day of surgery anesthesia was initiated with ether and maintained with isoflurane. Rats were fasted overnight. Single 25 mg/kg doses of racemic propranolol were orally administered. Simultaneous ECG and serial blood samples (200 µl/ sample) were then collected at 0, 15, 30, 45, 60, 120, 180, 240 and 360 min post dose. The catheter was flushed with 200 µl of saline after each sample collection. Samples were stored at –20° until analyzed for propranolol enantiomers using an HPLC assay.

Protein binding study:

The extent of propranolol plasma protein binding for adjuvant arthritis and control rats was calculated based on data generated in our laboratory [44] using equilibrium dialysis (Spectrum equilibrium dialysis apparatus, Los Angeles, CA). Protein binding of propranolol from individual adjuvant arthritis rats was measured *in vitro* by equilibrium dialysis for 4 h. at 37°C with Teflon half-cells separated by a spectra/Por cellulose membrane. Plasma was dialyzed against an equal volume of solution of propranolol racemate (10mg/L) in isotonic phosphate buffer (pH 7.4).

Before dialysis, plasma was adjusted to pH 7.4 by addition of 0.1 N HCl. These values were in agreement with Laethem *et al.*, 1995, which examined propranolol protein binding in inflammation.

Determination of the effect of anti-TNF therapy on propranolol pharmacokinetics and pharmacodynamics:

Sprague-Dawley rats were randomly divided into, control non-arthritic (n= 6) and arthritic groups (n= 7). When adjuvant arthritis was evident, both groups underwent the experimental protocol. The day before propranolol administration a silastic catheter was inserted into the right jugular vein. On the day of surgery anesthesia was initiated with ether and maintained with isoflurane. Rats were fasted overnight. On the day of the experiment, ECG electrodes, teflon coated wire, were placed in the dermis at the right and left axial regions and near the xyophoid process. Animals were allowed to recover for 2 h, and a baseline ECG was obtained. Single 25 mg/kg doses of racemic propranolol were orally administered. Simultaneous ECG and blood samples (200 µl/ sample) were collected at 0, 15, 30, 45, 60, 90, 120, 240 and 360 min post-dose. The catheter was flushed with 200 µl of saline after each sample collection. Plasma samples were stored at –20 °C until they were analyzed for propranolol enantiomers. ECG indices were obtained using the Acknowledge 3.01 data acquisition system. After completion of the protocol, adjuvant treated arthritic rats were dosed with a single *i.v.* dose of infliximab (3 mg/kg) and allowed to recover for 5 days. After completion of recovery time, anti-

TNF treated rats were dosed with propranolol again and the above procedure repeated.

Determination of the effect of NSAID therapy on Cardiac indices and propranolol potency:

Male Sprague Dawley rats were randomly divided into six groups. One day before the initiation of NSAID therapy baseline cardiac indices (PR interval and heart rate) were recorded for all rats. Rats were anesthetized initiated with ether and maintained with isoflurane. ECG electrodes, teflon coated wire, were placed in the dermis at the right, left axial regions and near the xyophoid process. Animals were allowed to recover for 2 h then baseline ECG indices were recorded. Each group received a specific dosage regimen; flurbiprofen (2.5 mg/kg bid *po*, 4 days), indomethacin (2.5 mg/kg bid *po*, 4 days), flurbiprofen and metronidazole (2.5 mg/kg + 50 mg/kg bid *po*, 4 days), metronidazole (50 mg/kg bid *po*, 4 days), celecoxib (5 mg/kg bid *po*, 4 days) and control (200 µl vehicle bid *po*, 4 days). At day two and four of the protocol, ECG indices were obtained, except for the metronidazole group for which ECG was recorded at baseline and day four only. At day four, after the ECG indices were obtained, rats were dosed with propranolol (25 mg/kg *po*) and response was monitored for 3 h.

Effect of flurbiprofen therapy on blood pressure and blood pressure control:

Male Sprague Dawley rats (300-350 g) were randomly divided into two groups (n=5/group), flurbiprofen (2.5 mg/kg bid *po*, 5 days) or control (200 µl vehicle bid *po*, 5 days). Within 12 h of the last dose animals underwent carotid artery cannulation. Silastic tubing was inserted into the right carotid artery and then attached to a pressure transducer (BIOPAC Systems Inc. Goleta, CA, USA), allowing for the determination of systolic and diastolic pressure. Baseline measurements were taken for 15 min. followed by administration of propranolol (10 mg/kg *ip*) and monitored for 3 h. A lower dose of propranolol was used due to the compromised sedated state of the animal.

Statistical Data Analysis:

A two-tailed Students t-test was used to determine statistical significant difference between any two groups. Determination of significance between multiple groups was done using ANOVA and a Posthoc Tukey. Linear regression was used to test relationships between percent change in PR interval and heart rate and dose, and as well as AUEC and dose following a range of propranolol doses. A two-sided α priori $\alpha = 0.05$ was used for all hypotheses tested. Data were expressed as mean $\pm$ standard deviation.

Propranolol Pharmacokinetic Data Analysis:

The area under the plasma concentration (AUC) was determined using the linear trapezoidal method. Elimination rate constants (β) were calculated using the regression slope of the log-linear terminal elimination phase. Assuming complete absorption, the oral clearance (CL_{oral}) was calculated from Dose/$AUC_{(0-\infty)}$. $AUC_{unbound}$ was calculated multiplying $AUC_{(0-\infty)}$ by the unbound fraction for both inflamed and control rats. [44] Modeled propranolol concentrations vs. time curves were obtained using WinNonlin (Pharsight Corp., Mountain View, CA, USA) two-compartment model (without lag time), utilizing pooled R propranolol plasma concentrations.

Propranolol Pharmacodynamic Data Analysis:

In all ECG measurements an average of five PR intervals and heart rate were obtained at each time point. Area under the effect curves (AUEC) was determined using the linear trapezoidal method. E_{max} and EC_{50} was calculated using WinNonlin (Pharsight Corp., Mountain View, CA, USA) PK/PD linked model, with pharmacokinetic data (R propranolol) modeled to a two-compartment model and pharmacodynamic data (% change in PR interval) modeled to a simple Emax model.

Chapter IV: The effect of Inflammation on Propranolol Pharmacokinetics and Pharmacodynamics

Introduction

An increase in drug concentration is generally assumed to result in increased effect and toxicity. Several investigators have reported substantial increases in the plasma concentration of many drugs due to diminished clearance caused by inflammation. [7-12; 44; 45; 59] This reduction in clearance is often attributed to a diminished hepatic metabolism brought about by increased expression of pro-inflammatory mediators such as nitric oxide (NO) and/or cytokines. [52; 158; 159]. The effect of various inflammatory conditions on the pharmacokinetics of propranolol, a β-adrenergic antagonist, has been studied by many investigators who have all observed reduced clearance. [7; 9; 11; 44] However, the therapeutic consequences of the observed increase have not been examined. It has also been reported that inflammation modifies β-receptor function. We hypothesized that for propranolol, the inflammation-induced β-adrenergic receptor down-regulation may be compensated by the inflammation-induced increase in drug concentration. Hence, we compared the cardiac effect of propranolol in inflamed and control rats.

Research Plan

Objective:

Using the adjuvant arthritis rat model, we set out to determine the effect of adjuvant arthritis on propranolol pharmacokinetics and pharmacodynamics.

Results

Effect of adjuvant arthritis on propranolol pharmacodynamics:

Induction of adjuvant arthritis:

Ten to 16 days after induction of adjuvant arthritis, swelling of the hind and forepaws was observed. Adjuvant arthritic rats had an average arthritic index of 6 ± 2 indicating moderate inflammation.

Baseline Cardiac indices:

Control rats had a baseline PR interval of 0.039 ± 0.0018 sec and a baseline heart rate of 398 ± 48 bpm. Adjuvant arthritic rats had a significantly prolonged baseline PR interval of 0.042 ± 0.002 sec and a baseline heart rate of 402 ± 48 bpm.

Dose effect curve:

Figure 2 depicts maximum percent change in PR interval and heart rate. Across the dosage range of 3, 5, 10, 20 and 30 mg/kg, propranolol triggered significant change in heart rate and PR interval in both adjuvant arthritic and control groups. The heart rate vs. dose relationship was linear (r^2= 0.85 p= 0.025) but rather

flat (Figure 2). With respect to PR interval, the effect-dose relationship appeared linear in controls (r^2=0.95 p= 0.0046) but reached a plateau after the 10 mg/kg dose in adjuvant arthritic rats. Heart rate was affected by the same magnitude in both groups. The effect of propranolol on the PR interval was also similar between the groups up to the 20 mg/kg dose. At 30 mg/kg dose, however, adjuvant arthritic rats exhibited significantly less potency than the control group. Figure 3 depicts the AUEC for % change in PR interval and heart rate. The AUEC for % change in PR interval vs. dose relationship was linear (r^2 = 0.86 p=0.024) in control rats, but there was no relationship in the adjuvant arthritic rats (r^2 =0.3 p=0.33). The AUEC for % change in heart rate vs. dose relationship was linear (r^2 = 0.8 p=0.04) in control rats, but there was no relationship in the adjuvant arthritic rats (r^2 = 0.02 p=0.8) (Figure 3).

Effect of adjuvant arthritis on propranolol pharmacokinetics /pharmacodynamics:

Induction of adjuvant arthritis:

Ten to 16 days after induction of adjuvant arthritis, swelling of the hind and forepaws was observed. Adjuvant arthritic rats had an average arthritic index of 7 ± 3 indicating moderate inflammation.

Propranolol pharmacodynamics:

Single doses of 25 mg/kg propranolol brought about significant changes in PR interval and heart rate from baseline in both groups of rats (Table 3 and Figures 4 and 5). Propranolols' ability to prolong the PR interval in adjuvant arthritic rats

was, however, significantly curtailed (56% reduction) as compared to control rats (Table 3 and Figures 4 and 5). On the other hand, adjuvant arthritis had no significant effect on heart rate (Table 3 and Figures 4 and 5).

Propranolol pharmacokinetics:

In all animals propranolol demonstrated stereoselective kinetics; with R-propranolol having significantly greater plasma concentrations than S-propranolol (Table 3 and Figure 6). There were no significant differences between enantiomers with respect to half-life or elimination rate constant. In the adjuvant arthritic rats, oral clearance of both enantiomers decreased resulting in significantly greater AUC values (Table 3). Although significant for both enantiomers, this effect was more pronounced for the R enantiomer. As a result the enantiomer AUC ratio (R/S) drastically changed from 4.8 ± 2.8 in control to 11.9 ± 10.8 in adjuvant arthritis rats. These results were in agreement with previous studies. [44]

Table 3 Mean ± STD, pharmacokinetic and pharmacodynamic indices of propranolol following single oral doses of 25 mg/kg to control and adjuvant arthritic rats (n= 4/group).

	Control		Adjuvant arthritis	
	R	S	R	S
β, h^{-1}	0.49 ±0.37	NC	0.65±0.3	1.72 ±0.3
$AUC_{(0-\infty)}$, µg h/L	265±125	57.5±7.2#	16278±9800*	1761±622*
$AUC_{unbound}$, µg h/L	15.1±7.1	12.7±1.6	212±127*	194±68.4*
$CL_{(oral)}$, L/h	14.7±4.9	NC	0.27±0.15 *	2.14±1.00*
Max % Change in PR interval	27 ± 1		12 ± 4*	
AUEC for % Change in PR interval (% change min)	4508 ± 2406		2418 ± 2199	
Max % reduction in Heart rate	18 ± 9		17 ± 4	
AUEC for % Reduction in Heart rate (% change min)	5058 ± 2128		3237 ± 509	

* significantly different from control.
NC – Not calculated, data below assay sensitivity
$AUC_{(0-t)}$

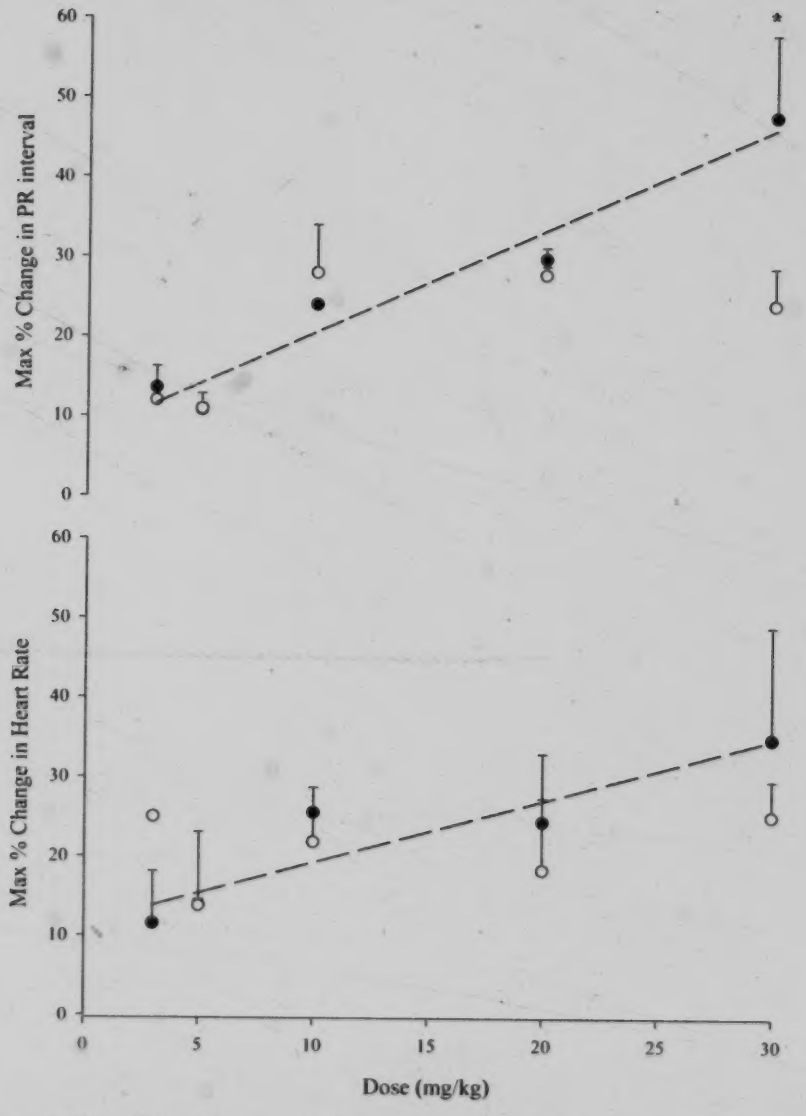

Figure 2 The effect of a range of propranolol oral doses on PR interval and heart rate in control (•) and adjuvant arthritic (?) rats (n= 3-4/group). Data points represent mean maximum % change from baseline. Error bars represent standard deviation; *significantly different from control.

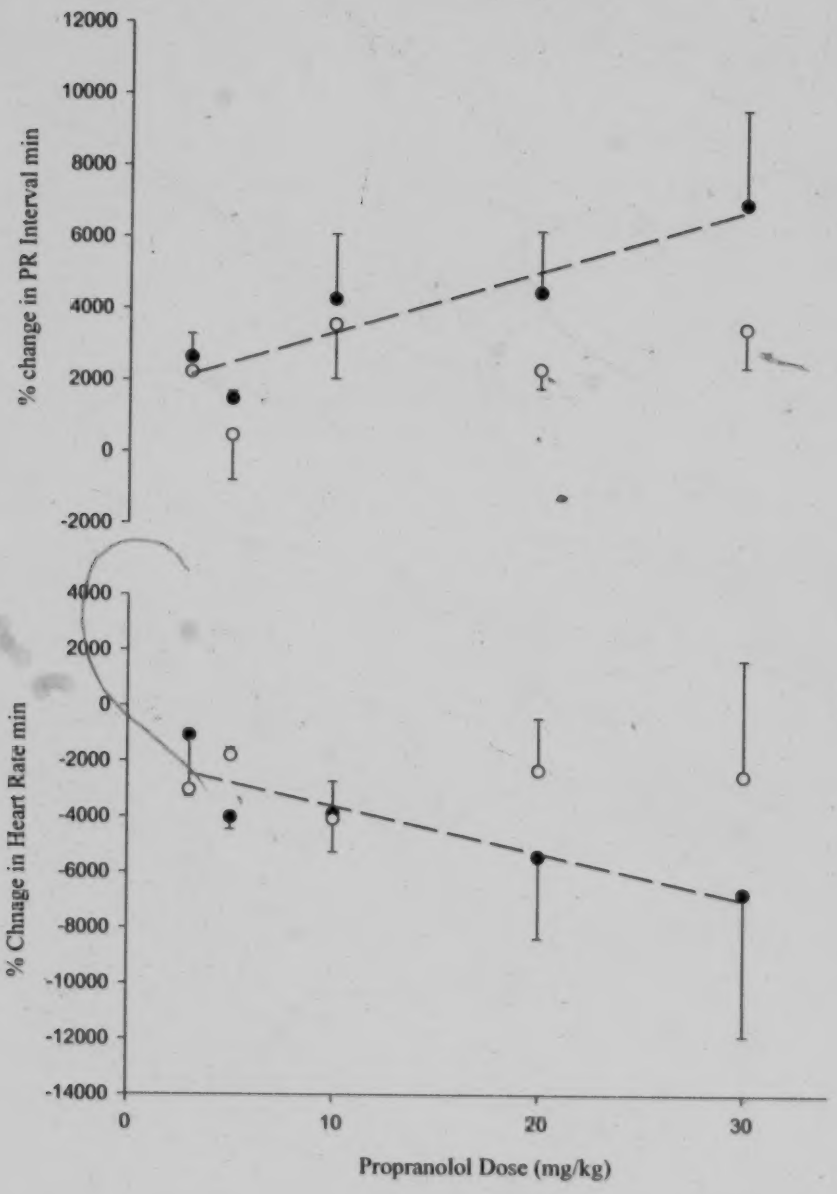

Figure 3 The effect of a range of propranolol oral doses on PR interval and heart rate in control (•) and adjuvant arthritic (?) rats (n= 3-4/group). Mean values ± STD.

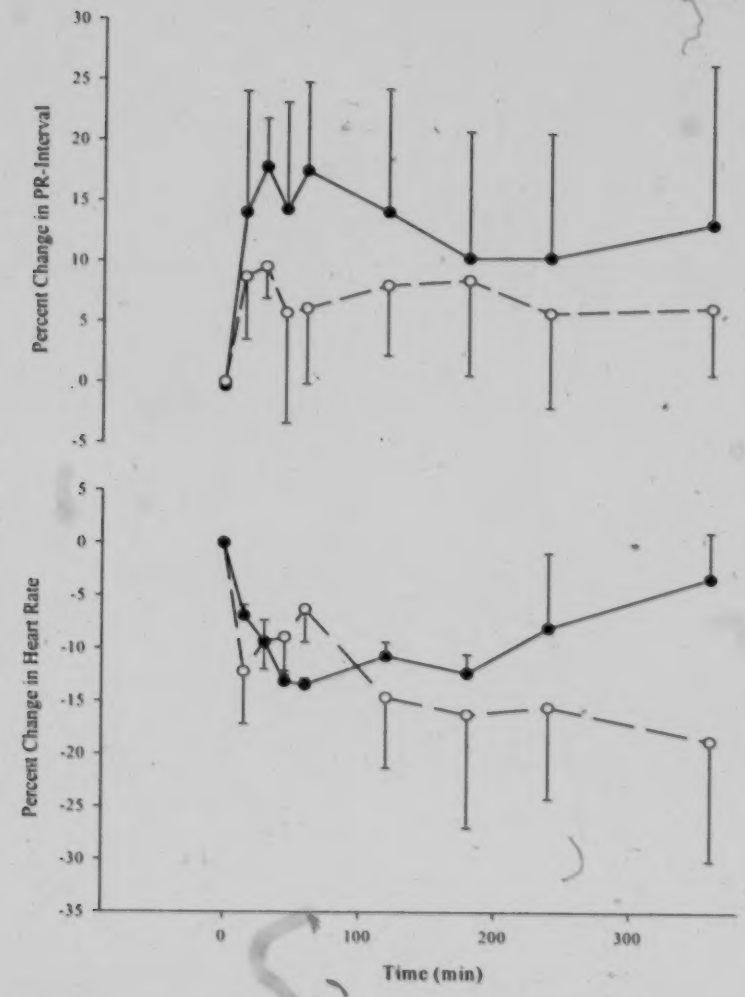

Figure 4 The effect of a single oral dose of propranolol (25 mg/kg) on percent change in PR interval and heart rate in control (•) and adjuvant arthritic (?) rats (open circles). Mean values ± STD (n= 4/group).

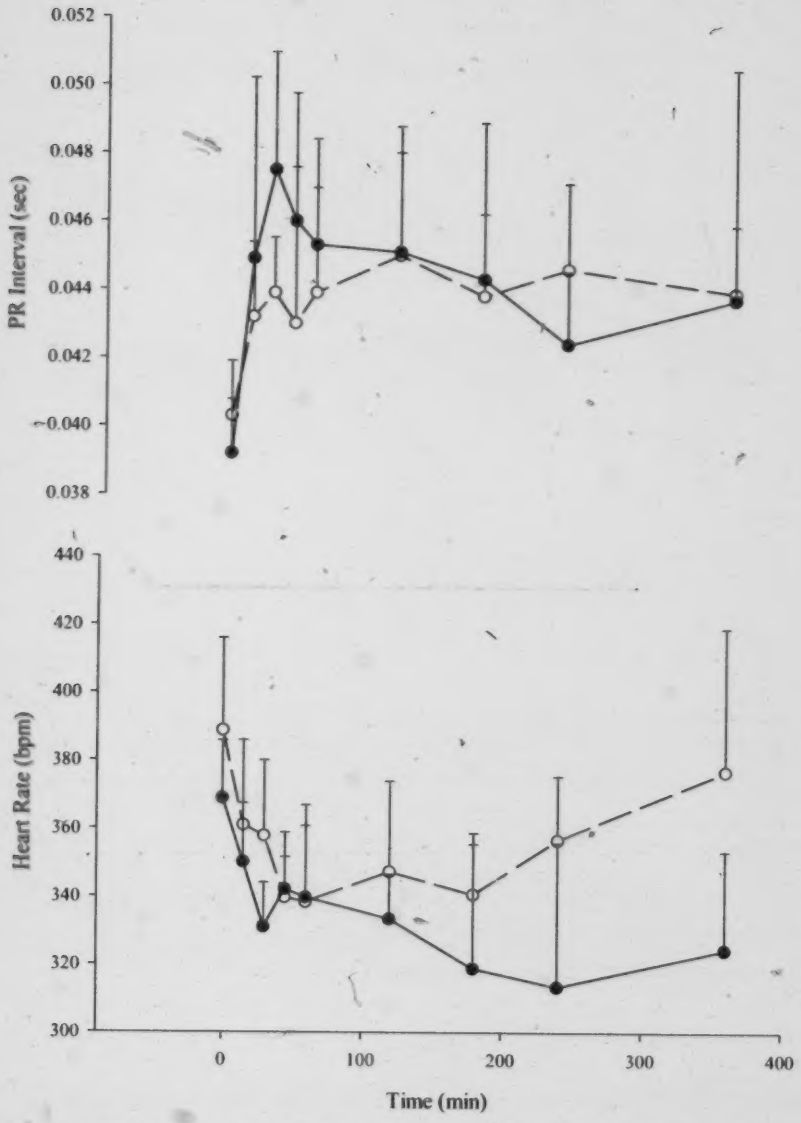

Figure 5 The effect of a single oral dose of propranolol (25 mg/kg) on PR interval and heart rate in control (•) and adjuvant arthritic (?) rats (open circles). Mean values ± STD (n= 4/group).

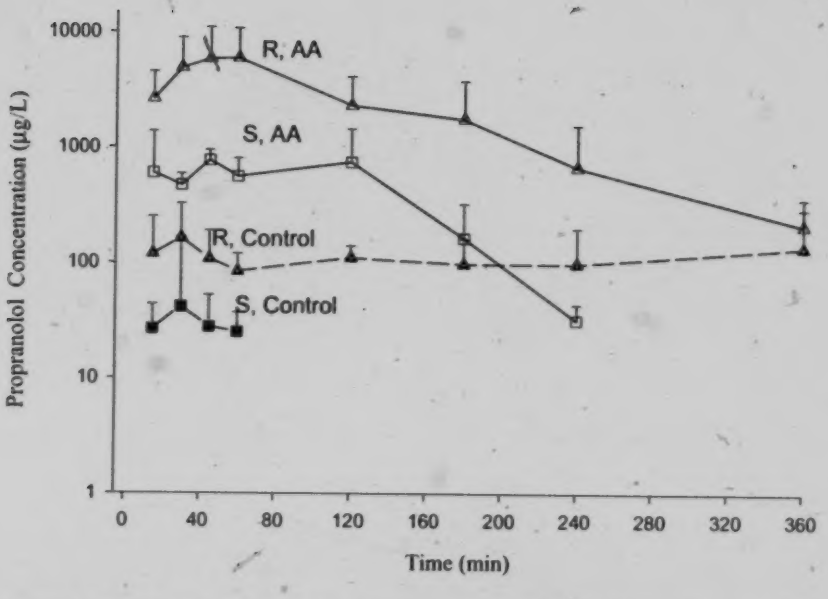

Figure 6 Concentration vs. time curves of propranolol enantiomers following oral administration of 25 mg/kg to control and adjuvant arthritic rats, mean values ± STD (n= 4/group).

Discussion

Previous studies have demonstrated a several fold increase in plasma propranolol concentration in adjuvant arthritis.[11; 44] This was confirmed by the present data. Inflammatory conditions such as adjuvant arthritis are associated with significant increases in the inflammatory mediators such as NO and TNF-α. [15] Several studies both *in vitro* and *in vivo* have shown that these mediators bring about a significant reduction in cytochrome P450 activity and content. [52; 159; 160] This triggers a reduction in clearance and a significant increase in propranolol concentration. Such an increase in plasma propranolol concentration is expected to result in increased potency and even toxicity. Interestingly, the several fold increase in propranolol concentration (Figure 6) resulted in no change or even reduced drug potency throughout the examined dosage range (Figures 2 and 3). With respect to PR interval elongation, there was an approximately 56% reduction in the effect at the 25 and 30 mg/kg doses (Table 3 and Figures 2-6) and no change within the 3-20 mg/kg dosage range in adjuvant arthritis as compared with controls. A reduction in the plasma unbound concentration (i.e., the concentration of pharmacologically active drug) or a modification of the site of action (i.e., receptor dysfunction) may explain these observations.

An inflammation-induced reduction in the plasma unbound propranolol fraction due to an increase in α_1-acid glycoprotein concentration may reduce the availability of the unbound drug to engage with receptors. [47] This, however, is an

unlikely explanation for our observation because despite the 50% reduction in the free fraction, the propranolol $AUC_{unbound}$ is still several folds greater in adjuvant arthritis as compared to control rats (Table 4). Hence, it is likely that inflammation alters receptor sensitivity.

The importance of α_1-acid glycoprotein binding in propranolol pharmacokinetics and pharmacodynamics was addressed by Yasuhara *et. al.* 1985 in a study examining the effects of laparotomy. In this study male Wistar rats were used as a model for postoperative inflammation and physiological change. Propranolol was administered orally (5 and 12 mg/kg) to control and laparotomized rats on day 2, 4 and 14 days after the operation. As well, propranolol was administered *i.v.* in control and laparotomized rats 2 days after surgery. Before drug administration blood samples were obtained for the analysis of urea nitrogen, creatinine, transaminase activity, total protein, albumin and α_1-acid glycoprotein. Propranolol pharmacodynamics was determined by determining the ability of propranolol to reduce an isoproterenol induced submaximal tachycardia. Anallysis of laboratory values revealed that total plasma protein was significantly higher two days after laparotomy. These results are consistent with those of Piafsky *et al.* (1978) and confirm that two days after surgery there is a surge of α_1-acid glycoprotein plasma concentration, it is believed that this increase is gradual and peaks at the 48 h period. [47] An examination of propranolol pharmacokinetics, after oral dosing, shows that laparotomized rats had significantly higher plasma concentrations. These results are consistent with those observed in our study and other studies. [11; 44; 54] However, after *i.v.* administration of propranolol a significant

decrease in elimination phase half life and volume of distribution was observed. Yasuhara *et. al.* argued that an increase in protein binding is responsible for the observed reduction in propranolol half life. These results are not consistent with those observed by Piquette-Miller and Jamali (1993).[44] According to the well-stirred model, changes in systemic clearance of highly extracted drugs is dependent on flow. Thus, reductions in propranolol systemic clearance could not be observed unless hepatic blood flow modified. If protein binding was responsible it would have to change to such as extent that propranolol is no longer a highly extracted drug. This very theory was addressed in an *in vitro* study by Grariepy *et. al.* it was shown that even if α_1-acid glycoprotein plasma concentrations were increased to 10g/l (which is double that observed by Yasuhara *et. al.*), [161] propranolol would continue to be classified as a highly extracted drug with an extraction of 0.82 ± 0.7.[161] Thus it is likely that the observed modification in systemic clearance was independent of protein binding. Another finding of the Yasuhara *et. al* study was that propranolol AUC (positive slope) and free propranolol AUC (negative slope) showed a linear relationship with α_1-acid glycoprotein. They concluded that this was evidence of a relationship between α_1-acid glycoprotein plasma concentration and propranolol disposition after oral dosing. In several studies including preliminary work done by Schneider *et al.* (1981), a relationship was established with acute phase protein, which was measured by the erythrocyte sedimentation rate. [8]With inflammation, acute phase proteins such as α_1-acid glycoprotein are elevated. As disease severity increases, erythrocyte sedimentation rate increases and so did propranolol AUC. [8] By measuring arthritic index, Piquette-Miller and Jamali

(1993) also found a relationship between disease severity and propranolol AUC. [44] A_1-acid glycoprotein may serve as an indicator of the severity of inflammation and may not necessarily indicate the importance of plasma protein binding in propranolol disposition.

An examination of propranolol pharmacodynamics in laparotomized rats by Yasuhara *et. Al* also showed very interesting results. They showed that the ability of propranolol to reduce isoproterenol-induced tachycardia was significantly reduced in laparotomized rats. These observations are consistent, with the reduction propranolol potency, which we observed (Table 4). However, Yasuhara *et. al* failed to address the possibility that changes may have occurred to the β-adrenergic second messenger system, and concluded that these observations were due to increased protein binding. Using the values obtained from Yasuhara *et. al*, two days after laparotomy total plasma protein increased by approximately 6%, while propranolol AUC increased by 4 fold. Therefore increased protein binding cannot explain the observed reduction in propranolol potency.[49] Isoproterenol-induced tachycardia was not used in our study as it was deemed not truly an assessment of physiological state. Rather it measures the effects of propranolol in a drug induced hypertensive state.

Clinical and *in vitro* evidence has illuminated the effect of disease states on drug pharmacodynamics. For example, β_2-receptor activity in peripheral blood mononuclear cells of rheumatoid arthritis patients is significantly reduced. [162; 163] Similar studies using septic patients have also reported significant reduction in β-adrenergic receptor responsiveness. [80; 81] In general, *in vitro* introduction of

inflammatory mediators (e.g., IL-1, TNF-α and INF- α) has resulted in reduced responsiveness, affinity and/or density of β- adrenergic receptors. [70; 83-85; 164] These *in vitro* observations may also be attributed to an over-production of NO secondary to inflammation or introduction of other pro-inflammatory mediators. [165] However, the effect of cytokines and NO is difficult to differentiate due to inter-relationship between these mediators.

Lack of increased drug effects despite increased concentration cannot be explained by a ceiling effect (i.e., examination of the pharmacological effect at the plateau phase of dose-response effect). The dosage range used was within the ascending phase of the dose-response curve in the control rats (Figures 2 and 3). In addition, at the 30 mg dose level significantly and substantially less potency was observed in adjuvant arthritis as compared with the control rats (Figures 2 and 3). As well, an examination of the PR intervals obtained over the six hours showed that although the baseline was higher in adjuvant arthritis rats, the PR interval never obtained the levels achieved in the control rats (Figures 4 and 5).

The reduced propranolol potency observed in adjuvant arthritis is similar to that observed following administration of calcium channel antagonist, verapamil, to rheumatoid arthritis pateints.[166] Mayo *et al.*, (2000) found a significant increase in verapamil plasma concentrations, but this did not result in any significant increase in verapamil potency or toxicity. This suggests that both β-adrenergic receptor antagonists and calcium channel blockers may be affected in inflammatory conditions.

Thus, in addition to the well-known inhibitory effect of adjuvant arthritis on drug clearance [160], the disease appears to reduce sensitivity of β-adrenergic receptors. The changes in propranolol pharmacokinetics and pharmacodynamics are likely due to a combination of NO and/or cytokine mediated inhibition of CYP 450 and receptor activity. Our observation in the rat may have significant clinical relevance. For example, it has been observed that patients suffering from rheumatoid arthritis have a significantly higher incidence of cardiovascular disease. [2] As well, it has been shown that in post-myocardial infarction patients, inflammatory status is the main determinant of therapeutic failure [76] or mortality. [167] Thus greater considerations must be given to potential drug-disease interactions in patients with multiple disease states. As well, altered metabolism and/or pharmacokinetics should be more closely examined for their pharmacodynamic consequences.

Chapter V: The effect of Anti-TNF therapy on Propranolol pharmacokinetics and pharmacodynamics

Introduction

Presently it is estimated that 1-3% of the world population suffers from rheumatic disorders such as rheumatoid arthritis. Rheumatic disorders significantly affect patients' quality of life and are associated with reduced mobility and increased pain. Rheumatoid arthritis patients, which are treated with a variety of anti-inflammatory agents, ranging from NSAIDs to, more recently, anti-cytokines agents (etanercept and infliximab).

In several studies it has been shown that rheumatoid arthritis patients' morbidity and mortality is significantly affected as compared to the normal population. It has been shown that patients with arthritis have a greater likelihood to suffer from angina, hypertension, and cardiovascular diseases. [2 3] It has been noted by physicians that those suffering from chronic rheumatic disorders have a reduced blood pressure control. [4]

We have shown, using inflammatory rat models that inflammation brings about significant modifications in propranolol pharmacokinetics and pharmacodynamics. Propranolol clearance is significantly reduced resulting in a several fold increase in circulating plasma drug concentrations. [43] Surprisingly though propranolol toxicity was not observed. Rather a reduction in propranolol potency was observed. [168] An examination of the literature points to the involvement

of inflammatory mediators such as cytokines (e.g., TNF) in the modifications of drug pharmacokinetics and pharmacodynamics.

In order to elucidate the extent of TNF involvement in the observed modification of propranolol pharmacodynamics and pharmacokinetics we decided to treat adjuvant arthritic rats with anti-TNF. In the past it has been noted that anti-TNF therapy does have significant affect on the severity of adjuvant arthritis. [137] As well, recent studies have shown that anti-TNF therapy is clinically effective in patients with both rheumatoid arthritis and Chrons' disease. [42] Using anti-TNF soluble antibody (infliximab) in a preliminary study we examined the ability of anti-TNF therapy to reverse the effects of adjuvant arthritis on propranolol pharmacokinetics and pharmacodynamics. These preliminary results would allow us to conclude if TNF is involved in the propranolol pharmacokinetic and pharmacodynamic changes.

Research Plan

Objective

Using the adjuvant arthritis rat model, determine if anti-TNF therapy can bring to normal the effects of adjuvant arthritis on propranolol pharmacodynamics and pharmacokinetics.

Results

Effects on propranolol pharmacokinetics

S propranolol plasma concentrations were below assay sensitivity in the control and anti-TNF treated groups and therefore none of the pharmacokinetic indices for S propranolol were determine in these groups.

Adjuvant arthritic rats demonstrated significant increase in AUC for the R enantiomer. S propranolol plasma concentrations were increased to levels that were detectable by the assay (Figure 7). In adjuvant arthritic rats R Propranolol AUC_{0-t} (9780 ± 5630 μg h/L) was significantly increased as compared to control (1150 ± 470 μg h/L).

Five days after anti-TNF treatment maintaince of jugular vein cannula was difficult, therefore only three samples were obtained in each rat. Pooled data allowed for pharmacokinetic modeling using WinNonlin (Figure 7). AUC_{0-t} was obtained for each rat and used for statistical comparison. With respect to R propranolol, anti-TNF treatment resulted in a reduction of AUC_{0-t} (6380 ± 1480 μg h/L). S propranolol concentrations following anti-TNF treatment were reduced to levels that could not be detected by assay.

Concentration vs. time curves for R propranolol for all three groups were tested using a repeated measure ANOVA to determine significance over the entire time period. Control concentrations were found to be significantly different from both adjuvant arthritic and anti-TNF treated rats (Figure 7)($p = 0.033$).

Effect on Baseline Cardiac indices:

Inflammation brought about an increase in PR interval in adjuvant arthritic rats (0.045 ± 0.005 sec) in comparison to control rats (0.041 ± 0.003 sec) but this trend did not reach significance. In the anti-TNF treated rats the PR interval was significantly reduced (0.038 ± 0.001 sec) in comparison to adjuvant arthritic rats ($p = 0.023$). Inflammation did not bring about any significant changes in baseline heart rate, with only a trend for increase seen in the adjuvant arthritic rats (389 ± 48 bpm) as compared to control (355 ± 45 bpm). Anti-TNF treatment (394 ± 72 bpm) did not significantly affect baseline heart rate although a trend for increase was seen.

Effect on propranolol pharmacodynamics

Propranolol potency was measured by looking the max % change in both heart rate and PR interval, as well as the % change in heart rate and PR interval AUCE curves (Table 4, Figures 8). Adjuvant arthritic rats had significantly reduced response to propranolol with respect to PR interval (Table 4). There was no significant difference in adjuvant arthritic rats with respect to propranolol's ability to reduce heart rate. Five days following anti-TNF treatment there was a significant change in propranolol potency. Anti-TNF treated rats had normal propranolol potency with respect to PR interval. The ability of propranolol to reduce heart rate was increased in the anti-TNF rats as compared to control (Table 4). An examination of the % change in PR interval and heart rate vs. time curves showed significant differences between the groups (Figure 8).

Using PK/PD link modeling, Emax and Ec_{50} values were estimated, these estimates show that in adjuvant arthritic rats there is a trend to a reduced Emax and increased EC_{50} (Table 5). With anti-TNF treatment there is a trend towards normalization of these values.

Effect on Inflammatory indicators

Arthritic index for adjuvant arthritic rats was significantly reduced five days following anti-TNF therapy (Figure 9).

Table 4 The effect of inflammation and anti-TNF treatment on propranolol potency with respect to PR interval and heart rate following a single oral dose (25 mg/kg) of propranolol.

Groups	Max. % change in Heart Rate	AUCE curve of % change in HR (% change min)	Max. % change in PR interval	AUCE curve of % change in PR interval (% change min)
Control	-20 ± 7	-38 ± 16	22 ± 8	59 ± 53
Adjuvant arthritis	-17 ± 18	-84 ± 24	-3 ± 15 *	-7 ± 38*
Anti-TNF treated	-26 ± 9	-94 ± 57 *	29 ± 17†	103 ± 53†

*Significantly different from control rats
†Significantly different from adjuvant arthritic rats

Table 5 The estimated propranolol Emax and Ec_{50} in control, adjuvant arthritis and anti-TNF treated rats.

Groups	Emax (% change in PR interval)	EC_{50} (μg/L)
Control	122	1.2
Adjuvant arthritis	27	1.7
Anti-TNF treated	43	1.4

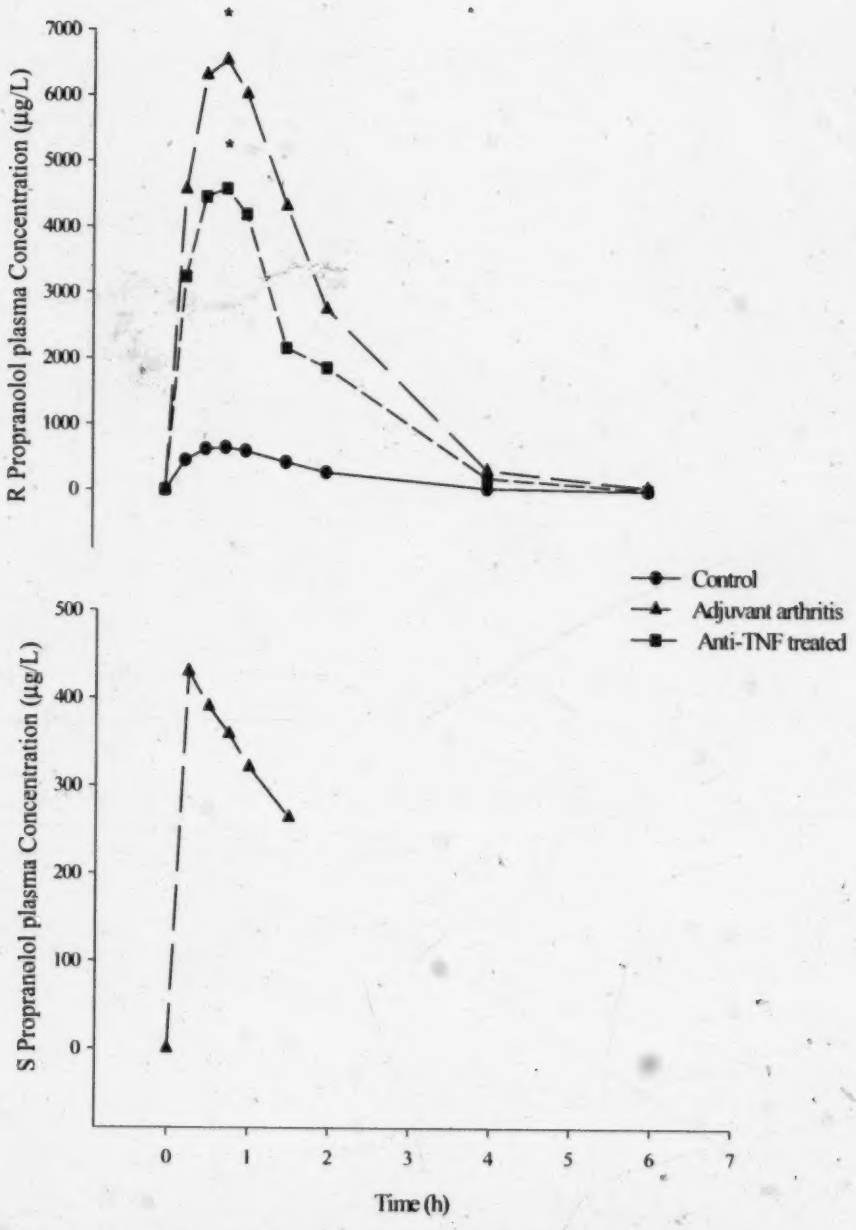

Figure 7 The effect of inflammation and anti- TNF therapy on R and S propranolol (25mg/kg PO). * Significantly different from control.

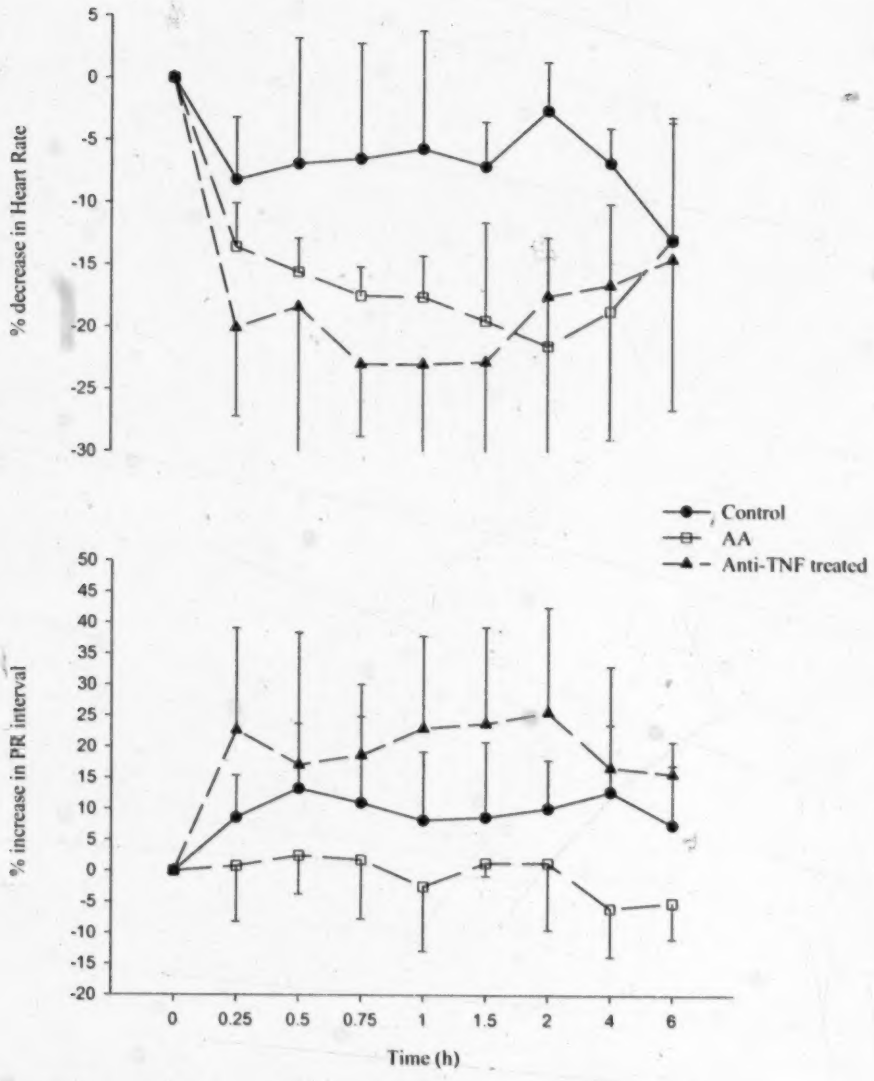

Figure 8 The effect of inflammation and anti-TNF therapy on propranolol potency (25mg/kg PO) with respect heart rate and PR interval.

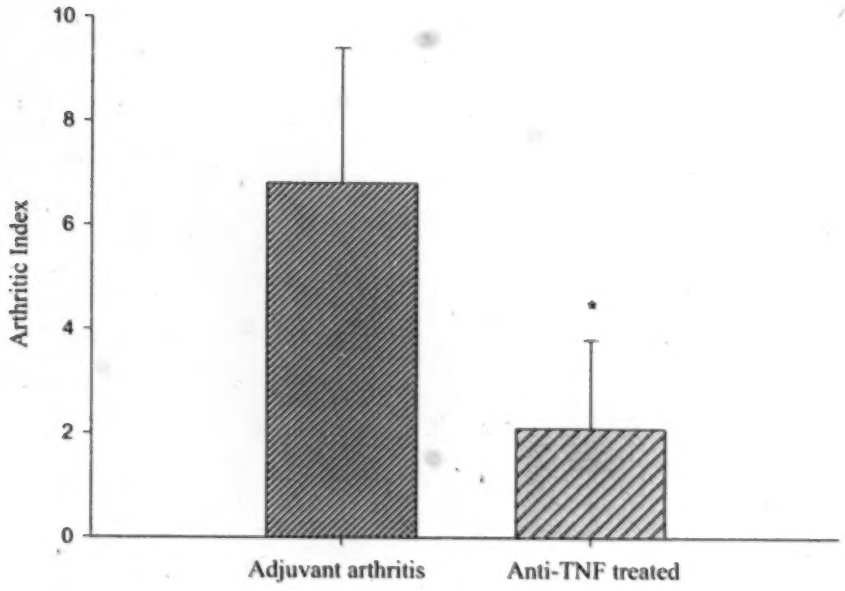

Figure 9 The effect of inflammation and anti-TNF therapy on arthritic index.

* significantly different from adjuvant arthritis.

Discussion

In this exploratory study, our data suggests that anti-TNF therapy returns to normal propranolol potency and, to some extent, reverses the effect of inflammation on propranolol pharmacokinetics.

Propranolol pharmacokinetics, as previously observed [43], was significantly altered in adjuvant arthritic rats. Adjuvant arthritic rats had a significantly elevated R and S propranolol levels (Figure 7). Following anti-TNF treatment to R-propranolol AUC was reduced to normal values and S propranolol levels were reduced to below assay sensitivity (Figure 7).

The effect of inflammation on propranolol pharmacodynamics, first elucidated by our group [168] was further confirmed by this study. Propranolol potency (i.e., max % reduction in PR interval) was significantly reduced in adjuvant arthritic rats (Table 4 and Figure 8). Anti-TNF therapy triggered a significant increase in propranolol potency as compared to adjuvant arthritis, with respect to change in PR interval. As well, anti-TNF therapy triggered increased propranolol potency with respect to reduction in heart rate, as compared to control (Table 4 and Figure 8). The effect of anti-TNF therapy on propranolol pharmacokinetics and pharmacodynamics confirms a possible role for TNF in inflammation induced physiological changes.

Inflammatory mediators act on the liver to modify CYP 450 function and activate acute phase protein synthesis. The result of CYP 450 modification is a significant reduction in drug clearance, as was observed in adjuvant arthritic rats

(Figure 7). This is believed to occur due to a dual process of direct interference of the CYP 450 heme group, occur by NO binding to the heme group. [43] The second mechanism, thought to be due to modification in CYP 450 transcription and translation.[52; 158]

The effect of anti-TNF therapy on propranolol pharmacokinetics confirms *in vitro* evidence linking TNF as well as other cytokines to changes in CYP 450 content and activity. [52] Five days after anti-TNF therapy, R- propranolol pharmacokinetics was brouhgt closer to normal values. Although anti-TNF therapy may bring about reduction in R-propranolol AUC this does not conclusively link TNF to changes seen in propranolol pharmacokinetics. Rather, anti-TNF therapy may merely be reducing disease severity, resulting in reduced modification in propranolol clearance. In a previous study by Piquette-Miller and Jamali (1995), a link between disease severity and changes in propranolol pharmacokinetics was established. Using female Lewis rats, inoculated with *Mycobacterium butyricum* to induce adjuvant arthritis. Untreated adjuvant arthritic rats and ketoprofen treated (2mg/kg daily), rats were assessed for severity and given an arthritic index ranging from 0-4 in each paw. Ketoprofen treatment was found to significantly reduce disease severity in those rats classified as severely arthritic. This was accompanied with a trend to normalization in propranolol pharmacokinetics. Further analysis showed that arthritic index and AUC_{0-8} demonstrated a linear relationship for both enantiomers (R propranolol $r = 0.79$; S propranolol $r = 0.8$). Thus with increasing severity an increase in AUC (i.e., more substantial reductions in CYP 450 activity)

was observed.[54] Thus by reducing disease severity, anti-TNF therapy may be triggering reduction in propranolol AUC.

The normalization of propranolol potency with anti-TNF therapy sheds light on the possible evolvement of TNF-α in inflammation induced changes in β-adrenergic receptors. Five days after anti-TNF therapy, propranolol potency, with respect to PR interval, was completely brought back to normal. Surprisingly as well, anti-TNF treated rats had significantly higher propranolol potency with respect to reduction in heart rate. Further, PK/PD modeling of data also shows that anti-TNF therapy partially restored both Emax and EC_{50} (Table 5). These results are consistent with clinical data results examining the potential use of anti-TNF therapy in treatment of heart failure. In patients with New York Heart Association class III to IV heart failure, anti-TNF therapy (etanercept 5mg/m^2 or 12 mg/m^2 SC twice a week for three months) resulted in a dose dependent increase in left ventricular function and improvement in patient functional status.[88] Thus with a reduction in plasma TNF-α, patients had significantly improved cardiac function.

The role of TNF-α and other cytokines in the modification of cardiac receptor function has been confirmed through *in vitro* studies and growing clinical evidence. It has been observed in our lab that even very acute exposure to cytokines (i.e., interferon-α 2a) resulted in a reduced sotalol potency.[169] TNF induced changes in calcium channels, possibly via the induction of NO and potentially other pathways, may be responsible for cardiac dysfunction seen in cardiac failure. The changes in calcium channels are thought to reduce contraction strength and thus triggering left ventricular dysfunction. Several studies have also shown modification

in β-receptor modifications with the exposure to inflammatory agents such as TNF-α.[70; 73; 91]

In several studies cytokine levels have been examined to determine if they may be biological markers for patient morbidity and mortality. In one of the largest analysis of cytokines and cytokine receptors that has been preformed to date, 1200 advanced heart failure patients (New York Heart Association class III or IV) were examined. Circulating levels of TNF, IL-6, soluble TNF receptor 1 and 2, soluble IL-6 receptor were measured at baseline and at the end of the study. It was observed that baseline levels of TNF soluble receptors and IL-6 were significantly higher in class IV heart failure as compared to class III. Further examination of survival rates showed they were related to levels of cytokines and cytokines receptors.[79] In patients with higher concentrations of circulating cytokines, survival rates were significantly lower. These data show a possible link between cytokines and patient outcomes. In a prospective randomized multicentre trail of low-molecular weight heparin use in post myocardial infarction, researchers had complied extensive data on troponin T, C-reactive protein and fibrinogen levels. A separate analysis of this data revealed startling relationships. Recruited patients presented with unstable angina or chest pain suggestive of acute myocardial infarction at hospital admissions. The mean duration of follow up was 37 months. In post-myocardial infarction patients, elevated C-reactive protein and fibrinogen, a general indicators of inflammation, were found to correlate with reduced survival.[170] Those patients that had an elevated inflammatory status were less likely to survive over the follow up period. Another study showed that plasma IL-6

concentrations could be used to predict the chance of future coronary events. Using only 43 patients admitted to coronary care unit due to unstable angina, the role of inflammatory cytokines in unstable angina prognosis was evaluated. It was found that patients with a higher IL-6 level at admission have a higher likelihood of having an in-hospital coronary event. [78] These studies demonstrate the link of cytokine levels to disease severity. Thus, routine cytokine concentration measurement may be viable predictive tool for disease prognosis.

Cytokines may also be indicators of long-term survival. In a very interesting study, a group of healthy elderly volunteers were monitored for the role of cytokine levels in long-term survival. IL-6 and C-reactive protein samples were obtained from1,293 Iowa, USA, residents that were otherwise healthy. Death certificates were used to measure survival rates over the ten-year length of the study. Researchers concluded that higher levels of IL-6 or C-reactive protein were associated with increased mortality. Subjects with elevations of both IL-6 and C-reactive protein levels were 2.6 times more likely to die during the follow-up period. [171] Regardless of medical condition, an elevated inflammatory status was an indicator of a reduced life span. These studies and others [172-174] clearly demonstrate that with increased inflammatory status patients have an increased likelihood of increased morbidity and mortality. In the future therapeutic guidelines may revolve around reduction in a patients' inflammatory status.

In our exploratory study we observed that adjuvant arthritic rats treated with anti-TNF had a significantly increased in propranolol potency with respect to reduction heart rate. These result although surprising may shed light on the role of

TNF in physiological homeostasis. It maybe that a reduction of TNF may trigger a rebound processes that sensitizes the receptors. Physiological similar phenomenon is observed with the sudden removal of β-adrenergic blockers and sertonin reuptake inhibitors. Thus with the removal TNF, which is acting as an inhibitory agent, there may be a rebound effect. Due to the ubiquitous nature of TNF, anti-TNF therapy may have far reaching effects, especially if taken for long term.

This pilot study set out to determine the effects of anti-TNF therapy on pharmacokinetics and pharmacodynamics of propranolol in adjuvant arthritic rats. Although we were able to answer a majority of our research questions, the study was limited by the several factors. Most notably, we failed to completely determine the effects of anti-TNF therapy on propranolol pharmacokinetics in adjuvant arthritic rats. Due to the nature of the adjuvant arthritis model, maintaining an indwelling catheter for five days proved impossible. By limiting blood sampling we were not able to robustly examine the effects of anti-TNF therapy on propranolol pharmacokinetics in adjuvant arthritic rats. As well, the adjuvant arthritis model prevented monitoring of anti-TNF effects past five days. This was compounded with assay sensitivity issues with respect to S-propranolol in control and anti-TNF rats. In both these groups the S-propranolol metabolism is extensive and S-propranolol concentrations are difficult to detect with the present assay. Thus, most of our results, although showing substantial changes in pharmacokinetics, were inconclusive. In future studies a different inflammatory model should be used, one with less dramatic physiological stresses on the animal. This would allow for a

better pharmacokinetic profile to be determined, as well as allowing for monitoring of anti-TNF therapy for a longer period of time.

Inflammation brings about substantial physiological changes in the body, including modification of drug metabolism and potency of some agents. We had previously observed that inflammation in the form of adjuvant arthritis brings about reduced propranolol clearance (Table 3). As well we had observed reduction in propranolol potency (Tables 3 and 4). The objective of this preliminary study was to determine if anti-TNF therapy would be able to reverse the effects of inflammation. We can conclude that anti-TNF therapy was able to reverse the effects of inflammation on propranolol potency. With respect to propranolol pharmacokinetics, although results were not complete, anti-TNF brought about normalization in R propranolol AUC. Although outside the scope of this pilot study, we also observed that anti-TNF therapy may have potential significant side effects. Anti-TNF treated rats had increased response to propranolol as compared to control (Table 4 and 5); the reason for this still remains unclear. Further work must be done to determine the effect anti-TNF on CYP 450 and drug pharmacodynamics in inflammatory models.

Chapter VI: The effect of NSAIDs on propranolol pharmacodynamics

Introduction

With an ever growing elderly population a large number of patients will experience co-morbidities and potentially serious drug and disease interactions. It has been observed that the elderly show reduced efficacy with regards to a variety of anti-hypertensives.[175] This has been hypothesized to either be due to physiological changes or a result of drug-drug or drug-disease interactions.[176; 177] One such interaction arises with the co-administration of NSAIDs and anti-hypertensives. [130]

NSAIDs act at the level of the cyclooxygenase enzymes. Traditional NSAIDs inhibit both cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1). [112] The COX-2 enzyme is activated during the inflammatory cascade; its inhibition curtails the inflammatory process. COX-1 is constitutively active producing prostaglandins needed for maintenance of physiological processes. Thus, the inhibition of COX-1 is fraught with potentially significant side effects, including reduced gastrointestinal (GI) membrane integrity and GI ulceration, which is preceded by GI inflammation. Thus, ironically, traditional NSAIDs initiate an inflammatory cascade due to modification in prostaglandin homeostasis. With the introduction of COX-2 selective agents, such as celecoxib, rofecoxib and meloxicam, GI toxicity may be reduced. [178]

The induction of inflammation is associated with several physiological changes. Previously our group has shown that patients with rheumatoid arthritis have reduced response to verapamil. [166] Using rat studies, we have shown that induction of adjuvant arthritis resulted in significantly reduced propranolol potency. Using this evidence, we hypothesize that the inflammation secondary to NSAID induced GI toxicity triggers reduced responsiveness to anti-hypertensives.

In this study we examined the effect of a number of traditional NSAIDs on cardiac indices (i.e., PR interval, heart rate and blood pressure). We also determined the effect of NSAID therapy on the propranolol potency to prolong PR interval and reduce heart rate and blood pressure. In addition, using metronidazole, an agent known to prevent NSAID induced GI toxicity [89; 179], and celecoxib, a COX-2 selective agent with limited GI toxicity[180], we tried to determine if GI toxicity would contribute to the effect of NSAIDs on cardiac indices and propranolol potency

Research Plan

Objective

Using Sprague Dawley rats, determine the effects of NSAID therapy on cardiac indices and propranolol potency and determine if these effects are linked to the prevalence of GI toxicity.

Results

The effect of NSAID therapy on baseline cardiac indices:

There was no significant change in baseline heart rate through out the four-day treatment in all groups (Figures 10 and 12). With respect to PR interval, a significant change in baseline values, in comparison to control, was observed after flurbiprofen and indomethacin therapy (Figures 11 and 13). A comparison within the flurbiprofen over time showed a significant change over the treatment period (Figure 11). Co-treatment with metronidazole (flurbiprofen and metronidazole) resulted in no significant change in PR interval over the treatment period (Figure 11). Treatment with celecoxib resulted in no significant change in PR interval baseline over the treatment period (Figure 13).

The effect of NSAID therapy on propranolol potency:

Propranolol potency with respect to PR interval (i.e., Max % change in PR interval and AUEC (% change in PR interval min) was significantly reduced following treatment with flurbiprofen and indomethacin (Table 6 and Figure 14). There was no significant change following treatment with celecoxib (Table 6 and Figure 14). Co-treatment with metronidazole (flurbiprofen and metronidazole) did not result in significant change in propranolol potency (Figure 14). With respect to heart rate, none of the NSAID treatments affected the Max % change in heart rate or AUEC, with respect to % change in heart rate vs. time. The AUEC (sec min) with respect to PR interval vs. time was not significantly affected by flurbiprofen therapy (Figure 16). With only a trend towards reduced AUEC being observed in

flurbiprofen (8.4 ± 0.6 sec min) and flurbiprofen and metronidazole treated rats (7.8 ± 0.7 sec min) as compared to control (8.3 ± 0.9 sec min). The AUEC (bpm min) with respect to heart rate vs. time was found to be significantly greater in flurbiprofen (58585.9 ± 4956 bpm min, $p < 0.05$) and flurbiprofen and metronidazole treated rats (56018 ± 3117 bpm min, $p<0.05$) in comparison to control (49832 ± 6713) (Figure 16).

Effect of flurbiprofen treatment on blood pressure, and blood pressure control:

Neither systolic nor diastolic baseline blood pressure was significantly affected by flurbiprofen therapy. Systolic baselines were 139 ± 15 and 129 ± 35 mmHg for control and flurbiprofen treated rats respectively. Diastolic baseline values were 119 ± 16 and 110 ± 22 mmHg for control and flurbiprofen treated rats respectively. As well, five day treatment with flurbiprofen did not significantly affect the ability of propranolol to reduce blood pressure (Figure 17).

Table 6 The effect of NSAID therapy on the AUEC of % change in PR interval and Heart rate. Mean values ± STD, * significantly different from control.

NSAID	AUEC % change in PR interval (% change min)	AUEC % Change in heart rate (% change min)
Control	2701 ± 1818	-1603 ± 2734
Flurbiprofen	249 ± 871*	-1332 ± 1458
Flurbiprofen and Metronidazole	1753 ± 1017	-1210 ± 591
Metronidazole	2241 ± 922	-743 ± 892
Indomethacin	-718 ± 1455*	-813 ± 1559
Celecoxib	2325 ± 1832	-919 ± 844

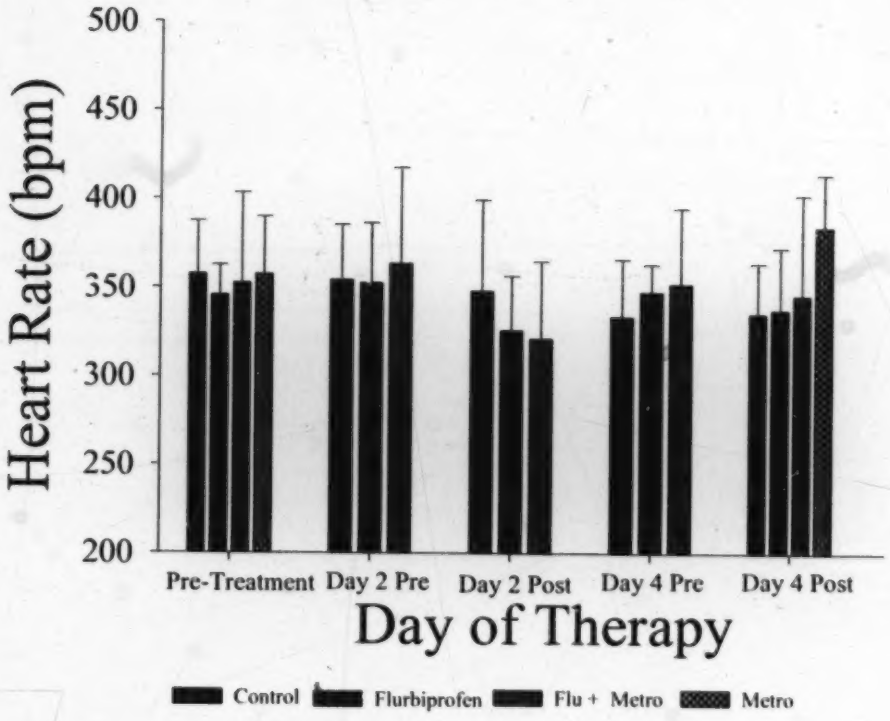

Figure 10 The effect of flurbiprofen therapy and co-treatment with metronidazole on the baseline heart rate (bpm), mean values ± STD. Animals were dosed with flurbiprofen once daily for four days and their ECG recorded before (Pre) and after (Post) dosing on days 2 and 4.

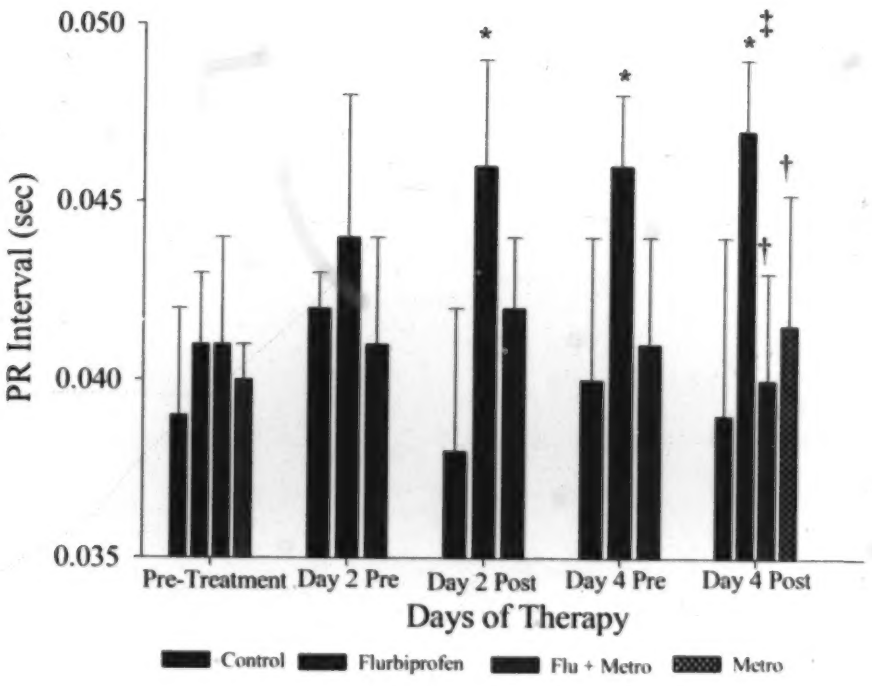

Figure 11 The effect of flurbiprofen and pretreatment with metronidazole on baseline PR interval (sec), mean values ± STD. Animals were dosed with flurbiprofen once daily for four days and their ECG recorded before (Pre) and after (Post) dosing on days 2 and 4.

* Significantly different from control ($p < 0.05$)
† Significantly different from flurbiprofen ($p < 0.05$)
‡ Significantly different from Indomethacin ($p < 0.05$)

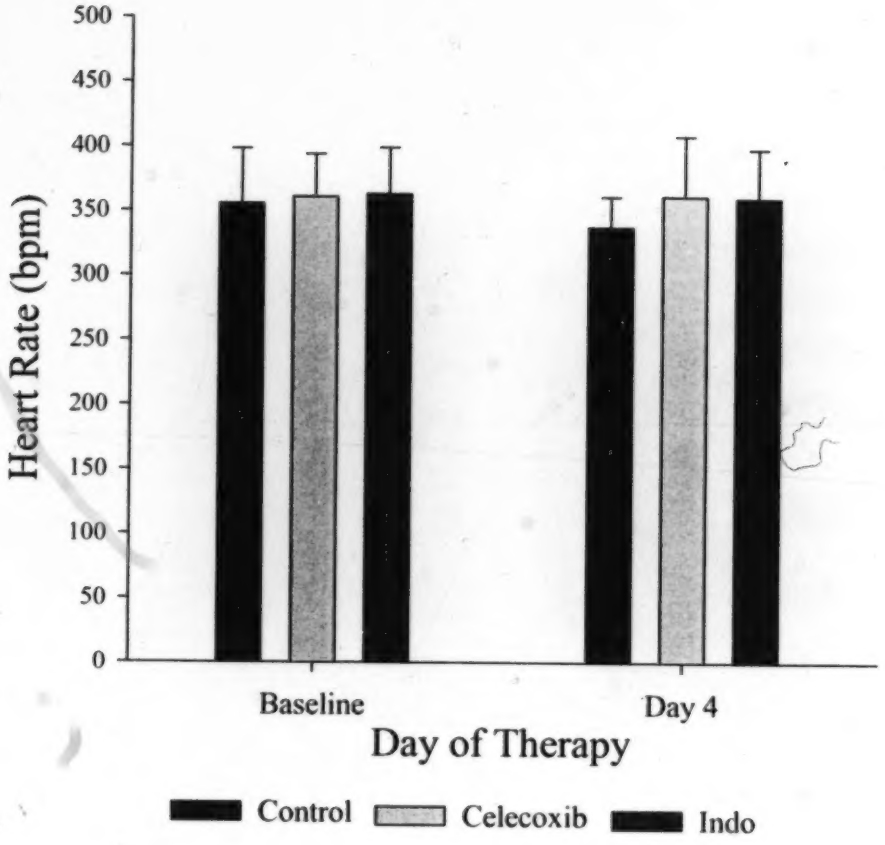

Figure 12 The effect of celecoxib and indomethacin therapy on baseline heart rate (bpm), mean values ± STD.

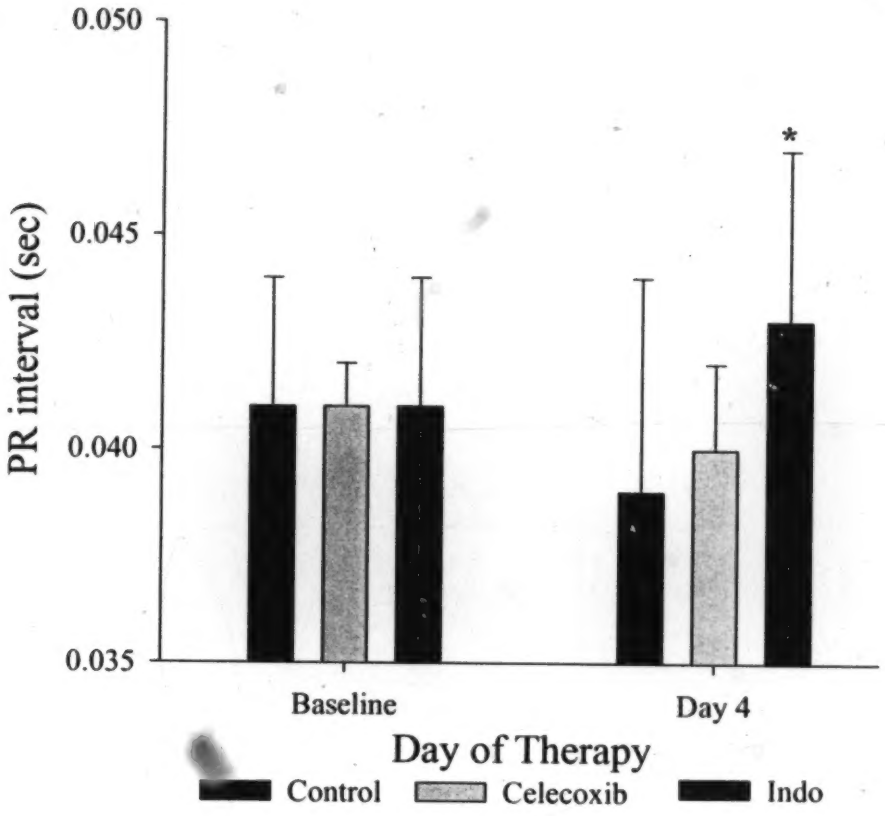

Figure 13 The effect of celecoxib and indomethacin therapy on baseline PR interval (sec), mean values ± STD. * Significantly different from control ($p<0.05$)

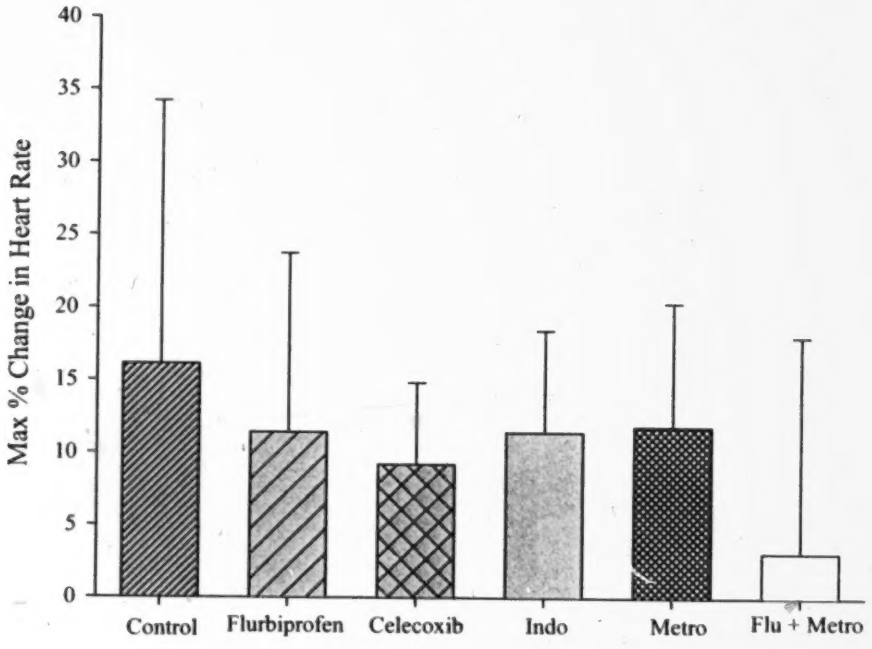

Figure 14 The effect of four day NSAID therapy (flurbiprofen, celecoxib, indomethacin, and metronidazole pretreatment) on the potency of propranolol (25 mg/kg *po*) with respect to heart rate, mean values ± STD.

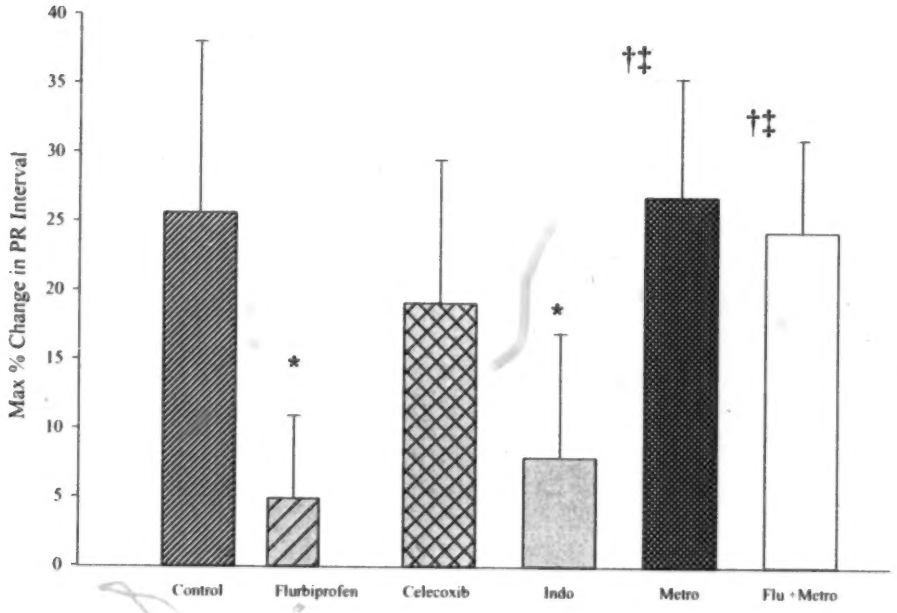

Figure 15 The effect of four days of NSAID therapy (flurbiprofen, celecoxib, indomethacin, and metronidazole pretreatment) on the potency of propranolol (25 mg/kg *po*) with respect to PR interval, mean values ± STD.

* Significantly different from control ($p<0.05$)
† Significantly different from flurbiprofen ($p< 0.05$)
‡ Significantly different from indomethacin ($p<0.05$)

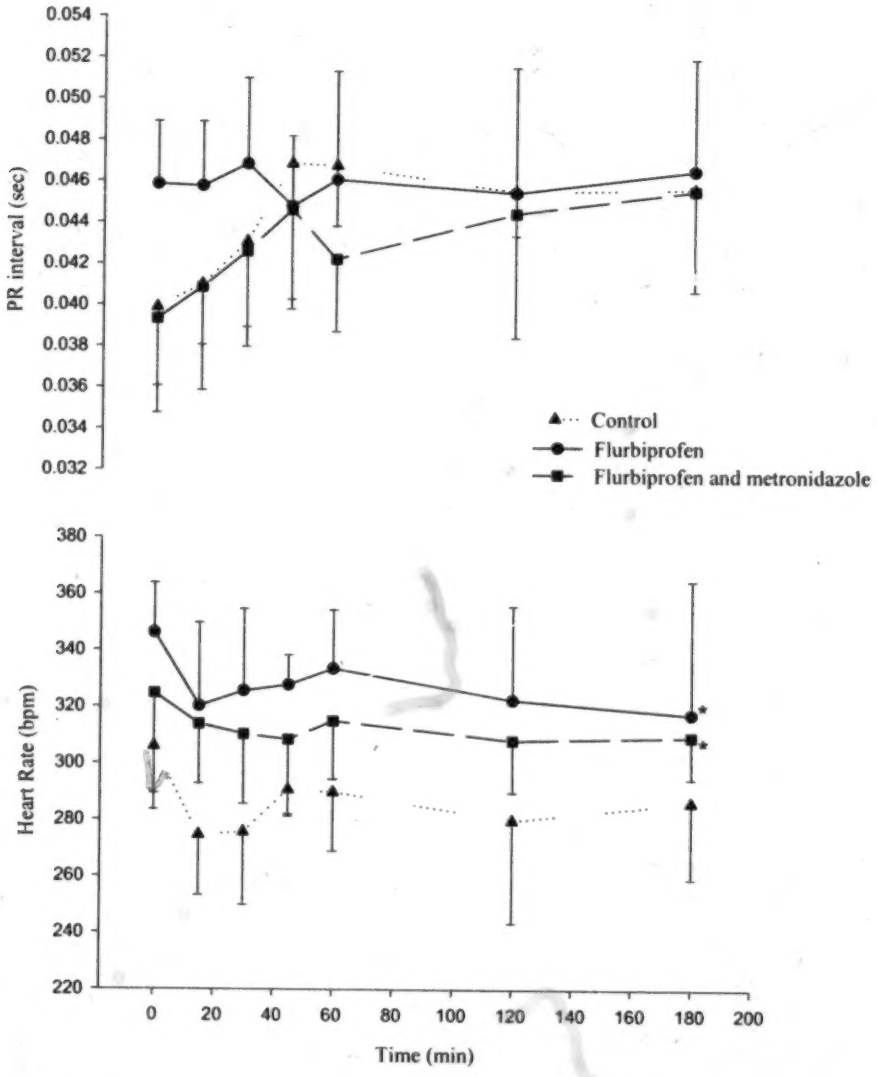

Figure 16 The PR interval (sec) and Heart rate (bpm) following administration of propranolol (25 mg/kg *po)* in rats treated with flurbiprofen, flurbiprofen and metronidazole and control rats. * AUEC is significantly different from control

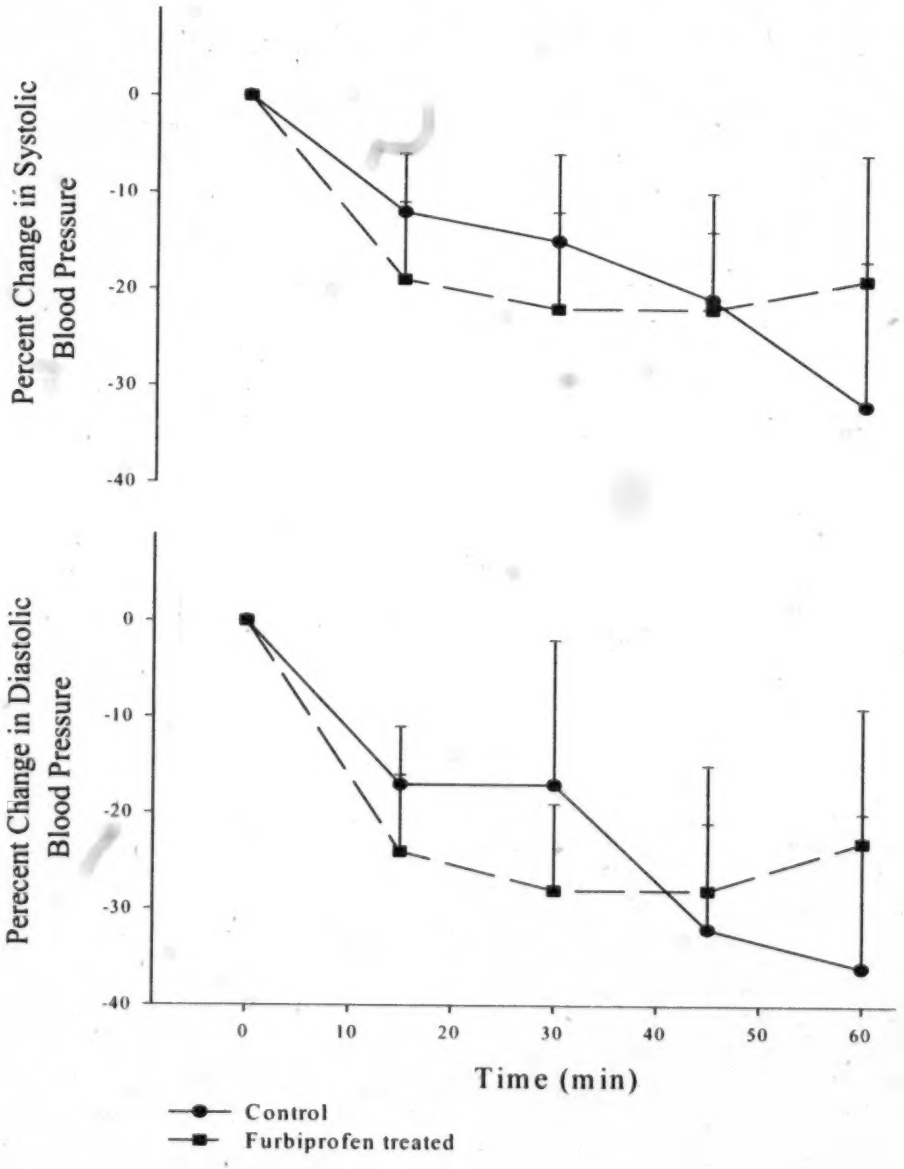

Figure 17 The effect of flurbiprofen therapy on the potency of propranolol with respect to systolic and diastolic blood pressure.

Discussion

In addition to the well known side effect of GI toxicity (i.e., ulceration and GI inflammation) NSAID therapy is associated with various side effects, including reduction in the effectiveness of anti-hypertensive drugs.[14; 130-132] In our study, rats treated with flurbiprofen and indomethacin had a significant prolongation in baseline PR interval over the treatment period (Figures 10 and 11). As well, a significant reduction in propranolol potency with respect to PR interval was observed in all rats treated with traditional NSAIDs (Table 6 and Figures 14 and 15). The modification in body homeostasis initiated by NSAID therapy may be responsible for the prolongation of baseline PR interval and curtailed propranolol potency observed in this study. Unfortunately the exact mechanism by which this occurs has not been examined in the literature.

Flurbiprofen and indomethacin therapy triggered a significant prolongation in baseline PR interval, with no change observed in heart rate or blood pressure. We tried to determine if this prolongation is associated with the effects of NSAIDs on the GI tract cell integrity. To do this we had two choices; misoprostol, a prostaglandin, is presently indicated for reduction and treatment of NSAID induced ulcers, or metronidazole. We did not use misoprostol because prostaglandins may have unknown cardiovascular effects and it does not prevent the formation of ulcers, rather it reduces the frequency. Instead, we used metronidazole shown by Davis and Jamali to prevent the formation of NSAID induced GI ulcerations. [118] When rats were given flurbiprofen 10mg/kg *IP bid* a significant increase in ^{51}Cr-EDTA

absorption (an indicator of GI ulceration). When rats were given metronidazole 50mg/kg *po* 12 hour before and one hour after flurbiprofen 10 mg/kg there was no increase in ^{51}Cr-EDTA absorption, thus proving that metronidazole prevents GI ulcer formation. [118] It is believed that metronidazole acts on the mitochondria reducing the formation of oxygen free radicals generated in mitochondria of GI cells, thus reducing cell turn over and maintaining GI integrity. [179] Using a modified protocol, we found that rats pretreated with both flurbiprofen and metronidazole had no effect on baseline PR interval. Thus by preventing the formation of GI inflammation, baseline PR interval was not modified. This is highlighted by the fact that when we used celecoxib, an agent with reduced incidence of GI toxicity, we also saw no change in PR interval baseline.

NSAID induced GI inflammation and ulceration brings about a significant increase in circulating inflammatory mediators, notably TNF-α. [181] TNF-α is believed to trigger significant cardiovascular side effects, through the disruption of Ca^{++} channel function. [182] Recent evidence points to a direct involvement in TNF and other cytokines in reduced heart function eventually leading to congestive heart failure and systemic edema.[88] TNF acting on Ca^{++} channels triggers reduction in strength of heart contraction, reducing ejection fraction and eventually leading to heart failure. By effecting a variety of ionic channels, its possible for an elevation in circulation cytokines to significantly affecting cardiac indices such as PR interval. We postulate that an NSAID induced increase in circulating inflammatory mediators such as TNF-α affect baseline cardiac indices (i.e., PR interval) (Figure 13).

Therapy with indomethacin and flurbiprofen triggered a significant reduction in propranolol potency (Table 6 and Figure 15). With the co-administration of metronidazole and flurbiprofen there was normalization of propranolol potency. Pointing to a link between the initiation of NSAID induced GI inflammation and reduction in propranolol potency. As stated earlier, we believe that a modification of receptor sites and ionic channels by inflammatory mediators such as TNF-α can affect baseline cardiac indices. Further, TNF-α has been shown to trigger reductions in β- adrenergic receptor affinity and/or density[83-85; 164] as well as modify the post receptor second messenger cascade. [70] TNF-α also triggers a significant increase in circulating nitric oxide which may also be contributing to the observed reduction in anti-hypertensive potency. [182] Thus, traditional NSAID therapy through its initiation of inflammatory processes (i.e., GI ulceration) brings about reduced anti-hypertensive potency by modification of the site of action. When we tested celecoxib, a COX-2 specific agent with reduced incidence of GI toxicity, no change in propranolol potency was observed. Thus further demonstrating that in the absence of GI toxicity no change of propranolol potency is observed.

An examination of the PR interval and heart rate over the 180 min following propranolol administration shows that flurbiprofen treated rats showed no response (i.e., curve is flat) to propranolol throughout the experimental period (Figure 16). This is in sharp contrast to control and flurbiprofen and metronidazole treated rats, which showed a noticeable change in PR interval and heart rate. These observations were further confirmed by the significant increase in AUEC, with respect to heart rate vs. time, after flurbiprofen treatment (Figure 16). Showing that flurbiprofen

treated rats had an elevated heart rate through out the study that was not reduced by propranolol therapy.

Traditional NSAID therapy is associated with a significant inhibition of both COX-1 and COX-2 activity in the kidney, resulting in reduced renal perfusion and function, thus reducing sodium and water absorption. This triggers an increase in blood volume, and is believed to be associated with as much as a 3-5 mmHg increase in blood pressure. Many researchers believe this to be the mechanism NSAIDs reduce the effectiveness of antihypertensives. [14; 130-132] In our study flurbiprofen therapy did not trigger any significant increase in baseline blood pressure (Figure 17). As well, the ability of propranolol to reduced blood pressure was not affected by flurbiprofen treatment (Figure 17). These results are consistent with findings of other researchers. [183] Harding *et al* 2000, found that therapy with indomethacin for 14 days brought about no change in baseline blood pressure in normal rats. [183] Rather, changes in blood pressure were only seen in a physiologically compromised animal, in this case rats that were sodium deprived. These findings can be attributed to the body's ability to adequately maintain blood pressure homeostasis in the absence of pathology. [183] As well, NSAID induced systemic water retention is usually associated with renal and cardiovascular pathology such as cardiac failure. Clearly, another mechanism by which NSAIDs affect the cardiovascular system exists.

In this study we examined the effect of celecoxib therapy on baseline cardiac indices (PR interval and heart rate) and propranolol potency. In both cases, celecoxib therapy did not trigger any change in either baseline cardiac indices or

propranolol potency (Figures 12- 15). These results were expected as second-generation COX-2 selective (e.g., celecoxib, meloxicam) and specific agents (e.g., rofecoxib) have reduced potential for GI inflammation, thus they would be less likely to increase plasma cytokine concentrations.[184] These results though seem to contradict recent studies examining the effect of celecoxib and rofecoxib therapy on blood pressure. In an *in vivo* rat study normal and hypertensive rats were dosed with celecoxib (10mg/kg) for three weeks. In both groups there was a significant increase in baseline blood pressure. [185] Celecoxib therapy did not cause any GI ulceration but a significant increase in leukocyte adherence was observed. [185] However these researchers did not examine PR interval or heart rate. As well, the length of therapy of their study limits extrapolation to our study, which was limited to four days of celecoxib therapy. Most notably researchers showed a significant increase in body weight with celecoxib therapy, this was not observed in our study and may be a major contributing factor to the observed increase in blood pressure. [185] In a recent study looking at celecoxib and rofecoxib in older hypertensive osteoarthritis patients, rofecoxib was shown to significantly elevate blood pressure as compared to celecoxib. In this randomized double-blinded trail, patients ≥ 65 years of age taking antihypertensive agents were either given celecoxib 200mg or rofecoxib 25 mg for six weeks. Patients had a greater tendency to develop edema and have elevated systolic blood pressure when taking rofecoxib as compared to celecoxib. [124; 125; 128] Thus, although COX-2 specific agents have significantly reduced incidence of GI toxicity, they seem to affect kidney function and may cause edema and exacerbate congestive heart failure.

In this study we examined the effects of NSAID therapy on propranolol pharmacodynamics, we did not examine the effects on pharmacokinetics. NSAID therapy has been found to significantly reduce the efficacy of anti-hypertensives; literature clearly associates this with a pharmacodynamic interaction. The potential for a pharmacokinetic basis for this interaction nonetheless should be addressed. In order for NSAIDs to reduce the efficacy of propranolol via pharmacokinetic processes two possible mechanisms may be occurring. Either there is an induction in propranolol metabolism reducing plasma concentrations, or a reduction in biologically active propranolol concentrations by increasing protein binding. An examination of the literature with respect to pharmacokinetic interactions between NSAIDs and other agents reveals that NSAIDs generally increases drug potency triggering clinically significant toxicity. [1] NSAIDs, generally acidic, are bound to albumin and have been associated with displacement of warfarin and pheyntoin from plasma albumin binding sites, increasing toxicity. NSAIDs are also not associated with any significant changes in CYP 450 activity, a study looking at metoprolol pharmacokinetics revealed that NSAID co-administration did not trigger any significant change in metoprolol pharmacokinetics. [1] Metoprolol is a highly extracted drug, which is hepaticly metabolized in a similar manner to propranolol. The inability of NSAIDs to affect propranolol pharmacokinetics, preculdes the examination of propranolol pharmacokinetics.

In this study we examined the effect of flurbiprofen therapy on systolic and diastolic blood pressure (Figure 17). We observed no significant change in baseline or the effect on propranolol potency with respect to their reduction. The study

involved dosing for five days, and thus was short term as compared to 14 [183] and 21 [185] day studies carried out by other researchers. The time line chosen was to maintain consistency with other parts of the experiment, which ran around 4 days long. In future, studies focusing on the effects of NSAIDs on systemic blood pressure should be longer in nature. As the effects of NSAID modifications on blood pressure homeostasis cannot be ascertained as quickly as those on PR interval and propranolol potency. This seems to show the presence of two distinctly separate mechanisms of action. An initial quick onset effect, acting on β-adrenergic receptors and calcium channels, due to increased plasma concentrations of inflammatory mediators; as well as a gradual effect occurring in the elderly and those with reduced kidney function, due to modification in kidney homeostasis.

Traditional NSAIDs, such as flurbiprofen and indomethacin have been known to significantly affect anti-hypertensives' potency. This was thought to occur due to the reduction in renal function. We propose another mechanism, linking NSAID induced increase in circulating inflammatory mediators, which can directly affect drug effectiveness (i.e.,anti-hypertensives). Therefore, not only patients with a history of renal or cardiovascular disease can be affected by traditional NSAIDs. Rather, patients that develop GI inflammation after using NSAIDs may be at risk of reduced anti-hypertensive control as well.

Chapter VII: Conclusion

The primary objective of patient care is to ensure that appropriate health outcomes are achieved. This may include everything from a reduction in toothache pain to blood pressure control. The means by which health care professionals and pharmaceutical scientists address these concerns is by focusing on the disease condition and eliminating physiological dysfunction. Obtaining the medication or discovering a molecule for the relief of an aliment is only the initial step in a long process. From a pharmacokinetic point of view, it is important that an appropriate concentration of the medication is achieved in the patient to allow a sufficient response, with limited side effects. What is often not addressed is the potential effect of innumerable environmental variables and physiological pressures which may have on drug pharmacokinetics and pharmacodynamics. Many of these variables can act as obstacles for achieving optimal therapeutic outcomes. The objective of this thesis work is to examine the effects of just one of these variables, inflammation.

The Effects of Inflammation on Propranolol Pharmacokinetics and Pharmacodynamics

The primary objective of this study was to determine the effects of inflammation on propranolol pharmacokinetics and pharmacodynamics. Using the adjuvant arthritis model, monitoring propranolol effects on heart rate and PR interval following several different propranolol doses, we were able to determine the extent of inflammation which modifies propranolol potency. In adjuvant arthritic

rats propranolol potency was significantly reduced across the dosage range tested (Figures 2 and 3), in spite of evidence for a significant elevation in propranolol concentration.[44; 54] In order to further confirm these results a simultaneous PK/PD study was conducted. In adjuvant arthritic rats a several fold increase in plasma concentrations of both propranolol enantiomers was observed, despite this a significant reduction in propranolol potency was observed (over 50%) (Table 3 and Figures 4-8). We believe that inflammatory mediators, activated during adjuvant arthritis, triggered a significant attenuation of drug metabolism and potency.

The intrinsic regulatory system of the body seems to have significant interaction with the immune system. Cytokines, activated during the inflammatory process, seem to be able act on virtually every part of the body. The question that arises is how do these agents, trigger these massive modifications in the body. Physiological examination of the cytokine cascade system shows several receptor types throughout the body. The receptor systems seem to directly act on DNA transcription and RNA translation, thus not only effecting immediate action of the cells, but also trigger long term effects.[186; 187] The observations we have seen are only a small part of the possible infinite effects of these agents.

The ramification of this research comes to light when examining a normal hospital patient population in which the majority of these patients have an elevated inflammatory status. Some, such as rheumatoid arthritis patients have a chronic state of inflammation; several studies have shown that in these patients a significantly higher rate of cardiovascular problems exists. In one study of 76 patients, aged 40-65 yr, diagnosed with rheumatoid arthritis were compared to 641

control patients recruited from the same North Glasgow area.[4] Patients and controls completed a questionnaire recording demographic data, medical history, drug history and social history. In rheumatoid arthritis patients the disease duration, presence of rheumatoid factor, health assessments as well as C-reactive protein and erythrocyte sedimentation rate were measured. In addition researchers also measured blood pressure, cholesterol, triglycerides, fibrinogen and other indicators of thrombosis. Rheumatoid arthritis patients had a significant increase in the rates of angina and non-specific chest pain, as well as a significantly higher diastolic blood pressure.[4] An examination of thrombotic variables also showed that rheumatoid patients have a significantly elevated fibrinogen and thus are more likely to have a thrombotoic event. In combination with a significantly elevated diastolic blood pressure, rheumatoid arthritis patients at significantly higher risk of cardiovascular disease. [4] These results, in conjunction with our observations, clearly show that the inflammatory status of rheumatoid patients clearly a predictor of the increased cardiovascular risk. With high levels of chronic circulation cytokines such as TNF, rheumatoid arthritis patients are likely to have modified β-adrenergic receptors, altered calcium channels and cardiac physiology. A close examination also reveals that rheumatoid arthritis patients have the perfect physiological milieu for cardiovascular disease to exist. This not only demonstrates that rheumatoid arthritis patients are more likely to have cardiovascular disease, but that cardiovascular disease has a significant inflammatory component. In the future therefore, cardiovascular disease therapy may be directed to not only treating the

symptoms of cardiovascular disease, but also alleviate the inflammatory component.[188]

Does anti-TNF Therapy Normalize Propranolol Pharmacokinetics And Pharmacodynamics?

In an effort to adequately understand the effects of inflammation we designed a pilot study to determine if anti- TNF therapy would result in normalization of pharmacokinetics and pharmacodynamics. Previous work had determined that reduction in diseases severity with NSAID therapy brought about a trend to normalization of pharmacokinetics. [54] By using anti-TNF therapy we wanted to observe if a reduction of adjuvant arthritis symptoms would normalize not only propranolol pharmacokinetics, but also pharmacodynamics. Anti-TNF therapy triggered a significant normalization of propranolol pharmacokinetics (Figure 7). This was accompanied with a significant normalization of propranolol potency with respect to elongation of PR interval (Tables 5 -6, Figures 8). Thus we observed that with a reduction in disease severity (Figure 9) we have a normalization of pharmacokinetics and pharmacodynamics. These results demonstrate a potential role for TNF in modifications of propranolol pharmacodynamics and pharmacokinetics. Further, these results reveal therapeutic alternative for not only rheumatoid arthritis patients, but also a variety of patients with systemic inflammatory conditions.

In adjuvant arthritic rats we had not only observed modified xenobiotic metabolism, but also a modification in the cornerstone of the sympathetic system

(i.e.,β-adrenergic receptors). Further examination showed that these changes were also observed in a variety of cardiovascular diseases such as congestive heart failure and post myocardial infarction. [66; 189] The normalization of propranolol potency, following anti-TN therapy shows that by removing inflammatory mediators and reducing disease severity we may be bringing about normalization of cardiovascular physiology. These results are further confirmed by clinical studies using anti-TNF therapy in patients with congestive heart failure and post myocardial infarct. These showed that with anti-TNF therapy left ventricle function was improved and excitation- contraction coupling was normalized. [88; 190] In a unique study looking at the role of TNF in a variety of congestive heart failure patients, it was found that a reduction in TNF levels, occurred with improving symptoms. When patients on conventional medication for congestive heart failure were monitored, it was found that as their symptoms were improved their plasma TNF levels were reduced. Those that failed to respond to therapy continued to have elevated plasma TNF levels. Thus, linking improvement in heart function with reduction in TNF levels. [191]

With anti-TNF therapy a sensitization to propranolol was observed; a significantly increased response to propranolol with respect to reduction heart rate. These observations, although preliminary in nature, nonetheless shed light on the potential role of TNF in cardiovascular homeostasis. Previous understanding of the cytokine system revolved on a balance between pro-inflammatory and anti-inflammatory cytokines; this relationship may not truly explain the full extent of this complex system.[186] This complex relationship can be most clearly illustrated with

studies looking at knockout studies. In IL-1β knockout mice, it was found that they lacked the ability to generate fever in response to local inflammation (turpentine induced) but still generated a fever when injected with endotoxin. More striking was the observed increase in average body temperature and reduced activity in IL-1β knockout mice.[186] It was believed that in the absence of IL-1β the body temperature would be reduced and the animal would not show symptoms of lethargy. Rather the opposite occurred, showing that IL-1β is needed to maintain body temperature homeostasis. These results point to a complex interaction between cytokines, where by the absence of anyone cytokine may disturb a fine balance.

Pathological processes are thus thought to alter this fine balance between various cytokines. Although anti-cytokine therapy is therapeutically effective, how does it affect this balance? Does it affect homeostasis? A recent study has shown that patients on anti-TNF therapy may develop tuberculosis. After all reports of tuberculosis, after infliximab therapy, were analyzed. It was found that the rate of reported cases of tuberculosis among patients treated with infliximab was higher than the normal rates. It was recommended that physicians should screen patients for latent tuberculosis infection or disease before prescribing infliximab. [192] Thus, anti-TNF therapy should continue to be monitored and caution exercised with long-term use. Due to the nature of this preliminary study we have been able to only speculate on the long term effects of anti-TNF therapy. Further work is needed to conclusively determine the effect of anti-TNF on the complex balance in the cytokine cascade.

The Effect Of NSAIDs On Propranolol Potency

NSAIDs are frequently used for the treatment of acute pain associated with inflammation. The concern with the chronic use of these agents is that they are fraught with side effects, which include GI ulceration. As well NSAIDs are noted for their interaction with antihypertensive agents. In this study we tried to determine if NSAID GI toxicity would alter the effectiveness of antihypertensives.

In our study we examined a variety of NSAIDs with differing ability to initiate GI toxicity. Flurbiprofen, indomethacin and celecoxib were tested, looking at changes in baseline cardiac indices and changes in propranolol efficacy with respect to heart rate reduction and PR Interval elongation. We observed that flurbiprofen and indomethacin therapy significantly affected baseline PR interval (Figures 10 and 11). No significant change in baseline cardiac indices was observed with celecoxib therapy (Figures 12 and 13). When flurbiprofen treated rats were co-treated with metronidazole, which prevents the formation of ulceration, a striking reversal was observed. With the prevention of GI toxicity there was no change in the PR interval (Figure 11). Further, we examined the effects of NSAIDs on propranolol potency with respect to heart rate and PR interval. Following four days of flurbiprofen and indomethacin therapy, propranolol potency was significantly reduced with respect to elongation in PR interval (Figure 15). The effects on propranolol potency were reversed with metronidazole co-treatment and absent in rats treated with celecoxib. Thus again we observed that in the absence of GI toxicity no change in propranolol potency occurs.

NSAID induced GI toxicity brings about an activation of the inflammatory cascade, which may contribute to the observed changes in the baseline cardiac indices and reduction in propranolol potency. In a recent study researchers looked at the time course of TNFα formation after indomethacin and flurbiprofen therapy in rats. It was observed that a single dose of indomethacin (10mg/kg *s.c.*) or flurbiprofen (30mg/kg) triggered a substantial increase in plasma TNFα in only 8 h, further this increase corresponded to increase in the intestinal damage score. This was also accompanied with increased iNOS activity. As well researchers observed that there was dose response relationship between indomethacin and increase in TNF α. Therefore with a single dose of NSAIDs, a several fold increase in TNF α was observed, due mainly to NSAID-induced GI ulceration.[181] In an *in vitro* study looking at the effect of NSAID therapy on cytokine production by human lymphocytes another mechanism was examined. Incubation of human lymphocytes with indomethacin triggered a significant increase in both mRNA and protein levels of TNF, IFNγ and IL-2. However, a down regulation in IL-4 and IL-6 was observed. These results further demonstrate that NSAID therapy may also have effects cytokine production through out the body. [156] With an NSAID-induced increase in plasma cytokines levels a significant alteration in β-receptor physiology and calcium channel activity may occur, as previously discussed. Thus patients with no history of cardiovascular disease and kidney dysfunction may still be at risk of reduced anti-hypertensive therapy.

Summary

In this study we tried to determine the effects of inflammation on propranolol pharmacokinetics and pharmacodynamics. Our results confirm the effects of inflammation on propranolol pharmacokinetics, and further shed light on the potential role of inflammatory mediators on the regulation of cardiovascular homeostasis. By modulating β-adrenergic receptor function, cytokines modified response to propranolol. By altering calcium channel function, cytokines may be responsible for changes in heart function and the observed elongation of baseline PR interval. In a pilot study we observed that anti-TNF therapy reduces disease severity and normalizes propranolol pharmacodynamics and to some extent pharmacokinetics. Most notably, we may be closer to understand the effect chronic exposure to cytokines can have on rheumatoid arthritis patients. We have shown in the adjuvant arthritis rat model that the entire drug response process is altered, and that chronic inflammatory conditions trigger unique physiological changes, which affect not only pharmacokinetics but pharmacodynamics as well. Therefore, pharmacokinetics cannot be used as the sole surrogate measure of drug response and a joint examination of pharmacokinetics and pharmacodynamics may be required to fully evaluate potential drug-drug and drug-disease interactions.

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